

T H E S I S

PART I

THE EFFECTS OF THE NATURE OF RESPIRED GASES
ON THE PULMONARY VASCULAR BED
WITH REFERENCE TO THE
EFFECTS OF CARBON DIOXIDE, OXYGEN AND NITROGEN

PART II

CHANGES IN RENAL FUNCTION DUE TO THE
ACTION OF CERTAIN DRUGS UPON THE HYPOTHALAMUS

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I. INTRODUCTION

It is the purpose of this thesis to discuss the effects of variations in the concentration of the normal constituents of the alveolar air on the pulmonary blood vessels and to describe some original investigations in this field.

Some of the difficulties in the investigation of pulmonary vasomotor activity in the intact animal have been discussed by Tigerstedt (1903), Wiggers (1921) and Daly (1933). One of the main problems is the need to consider the influences of variations in the cardiac output, systemic blood pressure and respiratory movements on the pulmonary blood vessels. If, for instance, the level of the pulmonary arterial pressure is taken as the criterion of the state of the pulmonary blood vessels, then this will clearly alter, not only with the calibre of the pulmonary blood vessels, but also with the cardiac output and the intrathoracic pressure. This latter is also important for its influence on the inflow to the right auricle and thus the right ventricular output. In certain conditions the pulmonary arterial pressure may be influenced by "back pressure" from the left auricle (see review by Daly, 1946). Events taking place within the lungs themselves may also have some bearing on the level of the pulmonary arterial /

arterial pressure, for it can be demonstrated that an increase of intra-alveolar pressure arising from bronchoconstriction can produce an increase of pulmonary vascular resistance (Daly and Hebb, 1942) and alterations in systemic arterial pressure may vary the amount of blood transferred from the bronchial circulation to the pulmonary circulation via the anastomoses between the bronchial arteries and pulmonary veins described by Miller (1940) and Berry and Daly (1931).

Pulmonary vasomotor activity can also be induced reflexly, for instance, by changes in left auricular pressure. Increasing the complexity of the inter-relationships is the observation that an increase of pulmonary arterial pressure may produce reflex cardiac slowing and that reflex changes in respiration can be produced by alterations in both systemic and pulmonary arterial blood pressures (Daly et al., 1942).

In the intact animal variations of the O_2 and CO_2 contents of the inspired air affect the cardiovascular and respiratory systems both directly and also reflexly. Any change in pulmonary vasomotor activity so observed may therefore be the indirect result of the various influences already described, as well as the independent effect of these gases on the pulmonary blood vessels.

It /

It is evident that from the above description that if it is wished to demonstrate that variations in the O_2 and CO_2 concentration of the inhaled gas mixture do exert any effect on the pulmonary blood vessels, then specialised techniques are necessary. Such methods should also exclude effects which might be produced on the pulmonary vessels through variations in humoral secretions. For instance, adrenaline, which has a direct action on the pulmonary vessels, is secreted during anoxia and asphyxia (Antrep, 1912; Kellaway, 1919), although Kellaway using the anaesthetised or spinal cat and the denervated iris as an indicator for adrenaline could find no evidence of excess adrenaline secretion during the inhalation of 5 per cent. CO_2 .

II. PREVIOUS WORK ON THE EFFECTS OF VARIATIONS
IN THE NATURE OF THE RESPIRED GASES
ON THE PULMONARY VASCULAR BED

Studies of the effects of variation of the alveolar concentration of oxygen and nitrogen on the pulmonary blood vessels date from the beginning of this century. Comparatively little work has been done on this aspect of pulmonary vasomotor activity; this is partly because the early results were confusing and partly because of the considerable technical difficulties involved.

Three main types of experimental approach have been used; as each has some inherent difficulties they will be discussed briefly:-

(a) The intact animal.- In the intact animal, severe operative procedures involving anaesthesia are required to demonstrate that variations in the concentration of the inhaled gas mixtures have any effect on the pulmonary blood vessels; even then it is difficult to make certain that the effects are not due to disturbances in the cardiovascular, respiratory or endocrine systems. The use of artificial ventilation, which is necessary to avoid effects due to variations in respiration, is liable to lead to an abnormal CO₂ concentration in the arterial blood during periods of administration of control gases.

(b) Direct observation.- The pulmonary blood vessels /

vessels have been observed by direct microscopy in the intact animal during periods of ventilation with test gas mixtures. In addition to the difficulties already discussed this method also suffers because only the subpleural vessels are visible. The subpleural capillaries have been shown to differ anatomically (Millar, 1940) from the alveolar capillaries and there can be no assurance that they react in a similar way.

(c) Isolated organs.— Changes in pulmonary arterial pressure and pulmonary venous outflow have been recorded following variations in the composition of the ventilating gas mixture in the heart lung and isolated lung preparations. These preparations suffer owing to the abnormality of the environment.

1. The Effects of Carbon Dioxide

Retzlaff (1913) found that, in cats under urethane anaesthesia and artificial ventilation, the inhalation of pure CO_2 produced an increase in the volume of a lung lobe enclosed in an oncometer. He regarded this as evidence of a pulmonary vasodilatation, but he did not consider the possible bronchoconstrictor action of high concentrations of CO_2 (Einthoven, 1892; Brodie and Dixon, 1903). Lohr (1924) was able to show in isolated cat lungs that the inhalation /

inhalation of CO₂ in concentrations of over 30 per cent. produced a bronchial spasm with increase in the volume of the lung, but CO₂ in lower concentrations led to bronchodilatation.

Von Euler and Liljestrand (1946b) found evidence of a pulmonary vasoconstrictor effect of CO₂ in intact cats under chloralose anaesthesia. The pulmonary arterial pressure was recorded directly with a Mellin's (1903) cannula. In their experience a change in the ventilating gas mixture from air to air containing 6.5 - 20 per cent. CO₂, produced an increase in both pulmonary arterial and systemic blood pressures, and had a negligible effect on left auricular pressure. Since these results were independent of the integrity of the vagi or the stellate ganglia or of the use of normal or artificial ventilation, they regarded them as evidence of a direct effect of CO₂ on the pulmonary blood vessels. However, the possibility that the pulmonary pressor response was influenced, at least in part, by cardiac and systemic vascular events cannot be neglected; indeed Logaras (1947), in similar experiments found that the effects of CO₂ on the pulmonary blood pressure were reduced or suppressed by ergotoxine which is known to reverse the effects of CO₂ on the systemic /

systemic blood pressure (Heymans and Bouckaert, 1933; von Euler and Liljestrand, 1946a). Wearn et al. (1926, 1934) who observed the pulmonary blood vessels in cats under sodium amytal anaesthesia by direct microscopy, did not find that CO₂ had any effect on the number of vessels open in the alveolus.

The heart lung preparation has not been found to be suitable for observing the effects of CO₂ on the pulmonary vessels, because ventilation of the lungs with gas mixtures containing high concentrations of CO₂ rapidly produces heart failure (Fühner and Starling 1913; Drinker, Churchill and Ferry, 1926).

In isolated lungs perfused through the pulmonary artery with heparinised blood at constant volume inflow, the inhalation of CO₂ produces an increase of pulmonary arterial pressure. This has been shown in the lungs of dog (Binet and Bourlière, 1941), Macacus rhesus (Hebb and Nimmo Smith, 1948) and cat (Nisell, 1948). Binet and Bourlière showed that the pulmonary arterial pressure was increased in proportion to the concentration of CO₂ inhaled. In isolated cats' lungs perfused at constant pressure with defibrinated or hirudinised blood, Lohr (1924) showed that CO₂ in all concentrations (from 1.4 to 100 per cent.) produced pulmonary vasoconstriction as shown by a decrease in blood flow. In his experiments, the effects /

effects of CO_2 on the vessels could be reversed if adrenaline (concentration $1:10^{-6}$ to $1.5:10^{-6}$) was added to the perfusate. Ketcham, King and Hooker (1912), observed that the inhalation of 10-25 per cent. CO_2 increased the rate of outflow from isolated rats' lungs perfused at constant pressure.

Carbon dioxide has also been shown to produce changes in the volume of blood in the lungs. Hochrein and Keller (1932), measured the flows in the pulmonary artery and pulmonary vein in the anaesthetised dog with Rein's thermostromuhr. They found that inhalation of 8 - 10 per cent. CO_2 led to a decreased flow in the pulmonary artery and an increased flow in the pulmonary vein. Sjöstrand (1935), also found evidence that the inhalation of CO_2 diminished the lung blood volume. He allowed mice to inhale approximately 20 per cent. CO_2 for some minutes and then, after the animals had been killed by decapitation, he removed and weighed the lungs. The weight of the lungs of these animals was significantly less than those of a control series. In the dog's isolated lung, however, Binet and Bourlière found that CO_2 inhaled in concentrations of less than 50 per cent. produced an increase of lung blood volume, and only when the concentration of CO_2 inhaled was more than 50 per cent. was the volume of blood in the lungs decreased.

2. The Effects of Oxygen

Neither Lohr (1924) nor Wearn et al. (1926, 1934) found that the inhalation of oxygen had any characteristic effect on the pulmonary vessels of the cat's lung. Retzlaff (1913) found that the volume of a lung lobe in an oncometer was diminished by oxygen inhalation and he, therefore, assumed that oxygen had a vasoconstrictor action. More recently, Von Euler and Liljestrand (1946b) have obtained evidence of a pulmonary vasodilator action of oxygen in the chloralosed cat. This effect is not abolished by ergotoxine (Logaras, 1947). Nisell (1948) has found that oxygen has a vasodilating effect on perfused cat lungs provided the ventilation is by negative pressure.

3. The Effects of Nitrogen

There is some recent evidence that inhalation of pure N₂ or gas mixtures containing a lower O₂ concentration than that present in atmospheric air may produce pulmonary vasoconstriction. Although previous investigators had found that nitrogen had no characteristic effect (Wearn et al., 1926, 1934; Retzlaff, 1913; Lohr, 1924). Motley et al. (1947) using a technique involving cardiac catheterisation found that the inhalation of 10 per cent. O₂ in N₂ for 10 minutes by unanaesthetised human volunteers increased /

increased the pulmonary arterial pressure and simultaneously decreased the cardiac output.

A pulmonary pressor response to the inhalation of 10 per cent. to 11 per cent. O_2 in N_2 has been obtained by von Euler and Liljestrand (1946b) in cats under chloralose anaesthesia. The effect was abrupt in onset and occurred simultaneously with a rise in systemic blood pressure although the left auricular pressure was unchanged. The response was not affected by stellate ganglionectomy or vagotomy, it was independent of the use of normal or artificial ventilation and was unchanged after the injection of ergotoxine (Logaras, 1947). Nisell (1948) obtained a similar type of response in two experiments on isolated cat lungs.

Pulmonary vasoconstriction of a different type has been observed by Dirken and Heemstra (1949a,b,c) in the rabbit. By ventilating each lung separately with gas mixtures of different O_2 content they were able to study variations in the blood flow through each lung which were reflected in changes in the O_2 saturation and O_2 tension of the carotid arterial blood. General anoxaemia could be prevented by a suitable choice of gas mixtures. If one lung (the "nitrogen lung") respired a gas mixture of low O_2 content (5-15 per cent. O_2 in N_2) and the other (the "oxygen /

"oxygen lung") a gas mixture of high O_2 content (60-99 per cent. O_2 in N_2), the pO_2 of the arterial blood fell initially but was gradually restored to the control value after approximately eight hours. This they interpret as indicating an establishment of pulmonary circulation in favour of the lung respiring the high O_2 mixture. They do not consider the possibility that there may be some diffusion of gas from one lung to the other through the visceral pleura. It is comparatively easy to demonstrate in the isolated lung that CO_2 rapidly diffuses through the visceral pleura, it would be expected that O_2 would diffuse less rapidly but the latent period for the response observed by Dirken and Heemstra (8 hours) might be long enough. However, Dirken and Heemstra were able to demonstrate that vasoconstriction did occur in the nitrogen lung by showing that it was pale in comparison with the other, that Evans blue injected intravenously spread more slowly to the nitrogen lung than to the oxygen lung and that the O_2 uptake from the nitrogen lung was reduced.

In view of the general lack of agreement between various workers as to the effects produced by variations in the concentration of the normal constituents of the alveolar air on the pulmonary vessels, it was decided that further work on the subject /

subject was justified. Isolated perfused lungs were chosen as the test preparation because in them conditions of circulation and ventilation can be absolutely controlled; it is also possible to exclude nervous and humoral effects on the vessels. It was considered important to control the conditions of the experiments by doing blood-gas analyses. This control is essential when it is wished to demonstrate the effects of O_2 inhalation, because only by an analysis of the O_2 content and percentage saturation of the blood during the control period can the possibility of anoxaemia be eliminated.

III. METHODS

The apparatus and technique used for the dog lung perfusions were as described by Berry and Daly (1931). A method was devised for perfusing cat lungs which was essentially a modification of that used by Daly and his co-workers for the lungs of other species. The apparatus used was constructed to provide a simple and easily assembled device for providing negative pressure ventilation, a constant volume inflow of blood and to protect the lungs from undue cooling and drying.

1. The Isolated Perfused Cat Lung Preparation

Cats weighing 2 to 4 kg. were anaesthetised with intraperitoneal chloralose (0.1 g./kg.) or nembutal (0.5 c.c./kg. Abbot's veterinary solution).

After a tracheal cannula had been inserted, the left carotid artery was cannulated and the animal was bled to death. The average amount of blood collected in this way was 33.1 c.c./kg. body weight (S.D. \pm 6.9 cc) Liquemin (Roche) was used as an anticoagulant. 1 c.c. (1000 I.U.) was injected into the external jugular vein before bleeding and the blood was collected into a measuring cylinder containing 1 c.c. of Liquemin. More Liquemin was added if more than 100 c.c. of blood were /

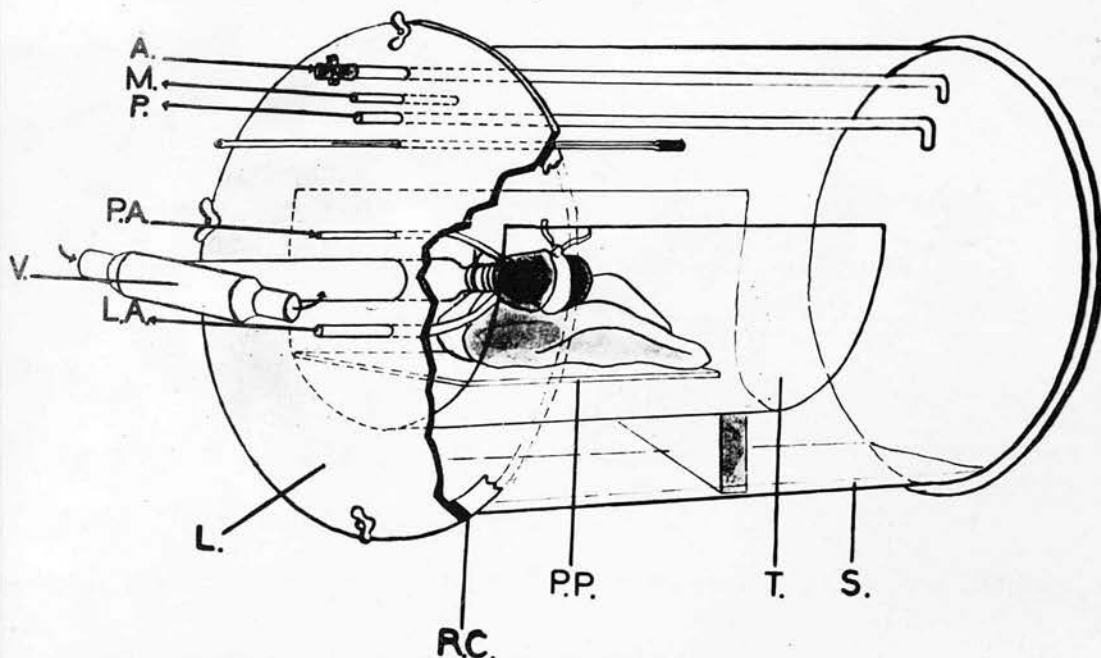


Fig. 1. Diagram of the apparatus used for the perfusion of isolated cat lungs. Part of the wooden lid has been cut away to show the position of the heart and lungs. A = AIR entry. P To Starling "Ideal" pump. M = Manometer registering pressure in chamber. PA = pulmonary artery. V = Respiratory valves. LA = Left auricle S = saline. T = Trough. L = lid. RC = rubber cushion. pp = perspex plate.

were collected.

Positive pressure ventilation was used throughout the period of bleeding and during the subsequent stages of the preparation.

After the animal's death the chest was opened, and ligatures were placed round the superior and inferior venae cavae and the thoracic aorta. The oesophagus was tied above the diaphragm and in the neck. Mass ligatures included all structures in the inlet to the thorax other than the trachea. The pericardium was opened and the pulmonary artery was ligated about 0.5 cm. from the ventricle. This ligature prevented air from entering the artery and it was removed before perfusion was begun. Cannulae were inserted into the pulmonary artery central to the ligature and into the left auricle. The ventricles were compressed with a tape.

All structures connecting the heart and lungs with the rest of the body were cut, and the heart and lungs and thoracic portion of the oesophagus were rapidly stripped off the posterior chest wall and transferred to the warmed respiratory chamber.

The respiratory chamber is illustrated in Fig. 1. It consisted of a cylindrical glass jar, 9 litres in capacity, placed on its side on a warmed operating table, and covered with an electric heating pad. The open /

open end of the jar was closed by a wooden lid which could be bolted into position by four wing nuts screwing on to a brass collar round the jar. A rubber cushion between the jar and the lid made this junction air tight. Tubes to the pulmonary artery, left auricle and trachea passed through holes in the lid, which were closed with rubber bungs.

The lungs were supported in a horizontal position in the chamber on a perspex plate resting on a perforated zinc trough. One end of this trough was fastened to the chamber lid, and a collapsible leg was fixed under the free end. Before the perfusion was started the lid with the attached trough was removed from the chamber, the lungs were placed in position and connections were made through the lid to the pulmonary artery, left auricle and trachea. The lid was then bolted into position and approximately 500 c.c. of saline at 35° C. were put into the chamber through a tube A (Fig. 1).

The perfusion apparatus.- The perfusion apparatus was essentially similar to that used by Berry and Daly (1932) for perfusing dog lungs. It consisted of a small Dale-Schuster pump, the output side of which was connected to the tube to the pulmonary artery, the venous outflow from the lungs was drained from the left auricle cannula into a venous reservoir, which, in its turn, was connected to the input /

input side of the pump. The total capacity of the pump and the connections to the lungs was 40 c.c. Tests of the pump showed that it maintained a constant volume output against a pressure change of 0 to 35 cm. blood. The pulmonary arterial inflow was 40 to 60 c.c./kg. depending on the size of the cat and the tone of the pulmonary blood vessels.

Recording apparatus.- The pulmonary arterial pressure was recorded by a tambour which could be directly calibrated by a manometer. The capacity of the recording apparatus was such that 0.25 c.c. of blood were taken up for each cm. blood rise in pulmonary arterial pressure.

Changes in the volume of blood in the venous reservoir were recorded with a volume recorder (capacity 10 c.c.). Since the lungs were perfused as constant volume inflow these changes show reciprocal changes in lung blood volume (Daly, 1928); although when variations in the lung blood volume are accompanied by changes in pulmonary arterial pressure, the apparent change in venous reservoir volume must be corrected for the volume change consequent upon the change in pressure.

Ventilation.- Negative pressure variations in chamber were made with a Starling "Ideal" pump. For this purpose a tube connected to the input of the pump was led into the chamber through a hole in the lid. /

lid. The pump was set so that it withdrew 250 to 500 c.c. of air out of the chamber 12 to 16 times a minute. The exact level of the negative pressure was shown on a water manometer and it could be varied by adjustments of a screw clip on another tube leading into the chamber (see Fig. 1). Air entered the chamber through this second tube to restore the pressure to atmospheric value on the upstroke of the pump. The lungs were, therefore, expanded by the negative pressure in the chamber and they collapsed by virtue of their own elasticity. In the majority of the experiments the extrapulmonary negative pressure varied from 0 to -10 cm. blood. In order to prevent evaporation from the exposed surfaces of the lungs the air in the chamber was kept moist and the air entry and exit tubes were led to the end of the chamber away from the lungs (see Fig. 1).

In a few experiments positive pressure respiration was used. In these the lungs were perfused in situ, and a Starling "Ideal" pump used for ventilation.

Temperature regulation.- The venous reservoir and the Dale-Schuster pump were immersed in a water bath at 38°C., the temperature of the blood in the pulmonary arterial tubing was thereby kept constant at 32.5°C. ($\pm 0.2^\circ\text{C}.$). The temperature in the chamber was approximately 30°C. The temperature of the lungs was purposely kept below the normal body temperature of /

of the animal, since it has been found empirically that dog's lungs are more resistant to oedema at low temperatures (Hebb, personal communication).

Administration of gas mixtures.- A T-piece containing Siebe Gorman inspiratory and expiratory valves was inserted across the tracheal cannula. A tube from the expiratory valve was arranged so that it could be connected at will to a small recording spirometer and in this way records of the expired air volume could be obtained for 3 to 6 respirations at frequent intervals. This figure is a measure of the tidal air volume and it was of the order of 30 to 45 c.c. at the beginning of the experiment. Douglas bags containing test gas mixtures could be attached to a tube connected to the inspiratory valve. It was so arranged that all the apparatus up to the inspiratory valve could be filled with the test gas mixture and that only approximately 10 c.c. of dead space remained between the valve and the bifurcation of the trachea. A closed respiratory circuit would have been of advantage because the tidal air could have been recorded continuously. However, this method was abandoned in favour of the one described above because it was not possible to keep the CO₂ concentration in the apparatus at a constant level. The gas mixtures used were all from commercial cylinders. No special precautions were taken to control the temperature /

temperature or the humidity of the mixtures, but since the Douglas bags were filled 10 to 20 minutes before the tests were made, this gave time for the gas mixture inside the bag to reach room temperature. Tests of the actual bags used, showed that they maintained a constant concentration of CO_2 over the experimental period for which they were required.

2. Dog Lung Perfusions

The method and apparatus used for the dog lung perfusions differed only in detail from those described above for the cat lungs. Dogs (8 to 20 kg. body weight) were killed by bleeding from the femoral artery, which was exposed under loval (Novocaine) anaesthesia, and the pulmonary artery, left auricle and trachea were immediately cannulated. The blood was collected and clotting prevented by the addition of heparin (B.D.H.) or Liquemin (Roche) 1,000 I.U./100 c.c.

The lungs were perfused with the animal's own blood through the pulmonary artery at constant volume inflow using a Dale-Schuster pump. The pulmonary arterial pressure and the volume of the blood in the venous reservoir were recorded, using similar apparatus to that described for cat lungs. In the majority of the experiments the blood inflow was adjusted to give an initial pulmonary arterial pressure /

pressure of 30 cm. blood, but in some experiments lower pressures were used (down to 7.5 cm.). The volume of blood in the venous reservoir was 500 to 700 c.c. at the beginning of perfusion and the circulation time was approximately 1 minute. The lungs were ventilated either by positive pressure using a Starling "Ideal" pump or by means of extrapulmonary negative pressure variations (ca.-2 to -12 cm. H₂O). When negative pressure ventilation was used the carcass of the dog with the lungs in situ was enclosed in a sealed chamber (of approximately 70 litres capacity), from which air was continuously removed by means of a "Vactrix" electric motor; a valve opening intermittently allowed the pressure in the chamber to be restored to approximately atmospheric value 12 to 18 times/minute. The expired air volume was recorded in the same way as has been described for cat lungs, it was of the order of 150 to 200 c.c. at the beginning of perfusion. The method of administration of gas mixtures has also been described above.

Four animals were anaesthetised with chloralose (0.1 g./kg.) before bleeding and of these two were kept alive by artificial respiration during the preparation for perfusion. The left auricle was cannulated, a ligature was placed round the pulmonary artery and the animal was bled from the carotid artery directly, through a filter consisting of glass wool soaked /

soaked in saline, into the venous reservoir. While positive pressure ventilation was still maintained, a cannula was inserted into the pulmonary artery and perfusion was begun. Perfusion was continued while the lungs were removed from the body and transferred to the negative pressure chamber. This method ensured that the circulation through the lungs was interrupted for only 2 to 5 minutes (instead of 30 minutes or longer).

Biochemical Estimations

Blood gas estimations.- In both dog and cat lung perfusions, samples of blood from the pulmonary artery were taken into small glass tonometers and stored over mercury. Analysis of the oxygen and carbon dioxide content of these samples was done by the method of Peters and Van Slyke (1932). The analyses were made 2 to 4 hours after the end of perfusion, during which time the tonometers were stored in a refrigerator.

Haemoglobin estimations.- Haemoglobin in blood samples was estimated as ferrous haem by the method of Stadie (1920) and Wu (1922) as described by Peters and Van Slyke (1932), using a Hilger Spekker photometer and a No. 5 filter. The Spekker was calibrated with blood of known O₂ capacity taken from dogs, cats, humans and oxen. The haemoglobin was calculated from the oxygen capacity assuming (vide Barcroft, 1934) that /

that 1 gm. of haemoglobin combines with 1.34 c.c. O_2 .

No variations in the concentration of haemoglobin in the blood were observed as a result of ventilation of the lungs with the various gas mixtures used.

In some experiments the percentage saturation of the arterial blood was calculated from the haemoglobin concentration and the oxygen content. This gave an approximate value only because, since no analysis of alveolar air was performed, no correction could be made for the amount of dissolved oxygen.

3. The Condition of the Cat Isolated Lung Preparation during Perfusion

Observations on the cat heart-lung preparation have shown that, when the perfusion is performed with defibrinated or hirudinised blood or blood to which Chicago blue has been added, pulmonary oedema frequently develops (Evans, 1912; Knowlton and Starling, 1912; Newton, 1932). Isolated cat lungs perfused with diluted defibrinated blood are also apparently susceptible to oedema (Bülbring and Whitteridge, 1945). It was therefore considered necessary to find out if cat's lungs were suitable preparations for perfusion experiments. Observations were made of the condition of the preparations both during perfusion and at the end of the perfusion, and
in /

in addition certain other tests were made (see below).

Four main criteria were used in assessing the condition of the preparations:-

(a) Pulmonary arterial pressure, lung blood volume and tidal air changes during perfusion

The initial pulmonary arterial pressure was often high, during the first few minutes of perfusion it was often as high as 30-40 cm. blood with a pulmonary arterial inflow of only 50 c.c./minute. Although the pressure tended to fall with continued perfusion and with the start of negative pressure ventilation, the rate of decrease of pressure was materially hastened by one or two positive pressure inflations of the lungs. In one experiment atropine (2.0 mg. atropine sulphate injected into the pulmonary arterial tubing) also appeared to have a beneficial effect.

The reason for this high initial pulmonary arterial pressure is obscure. It has been suggested (Bayliss and Ogden, 1933; Eicholtz and Verney, 1924) that shed blood contains vasotonins and bronchotonins which are removed during passage through the lungs. The rapid decrease in pressure which followed positive pressure inflation of the lungs suggests that some mechanical factor may also be involved, such as a cohesive force between the alveolar walls which would maintain a partial state of collapse of the lungs.

The /

The pulmonary arterial pressure was kept, if possible, below 30 cm. blood and the pulmonary arterial inflow was only gradually increased as the pressure fell.

After the first 10 to 15 minutes of perfusion and with constant volume blood inflow and constant artificial ventilation, the pulmonary arterial pressure and lung blood volume showed little change for the first 2 to 3 hours of perfusion. A slight but progressive increase of pulmonary arterial pressure and lung blood volume sometimes occurred after this period.

The lungs showed good expansion in inspiration and collapsed rapidly in expiration in the majority of experiments. The tidal air, however, showed the gradual progressive diminution which is usual in perfused lung experiments (Daly et al., 1946) Löhr (1924) perfusing cats lungs with defibrinated or hirudinised blood noticed an intense bronchospasm at the beginning of perfusion, this was not observed in the present series.

(b) The condition of the lungs at the end of perfusion

At the end of perfusion the lungs were removed from the chamber and were examined externally and after slicing for signs of oedema, congestion, atelectasis, bronchial exudate and peribronchial or /

or perivascular haemorrhage or other abnormality.

Each preparation was then classified as:+

- (i) very good: no visible abnormalities.
- (ii) good: abnormalities in one or two lobes only, and it was considered that these might have been the result of local mechanical influences.
- (iii) poor: several abnormalities in more than two lobes.

Of 26 experiments 7 were classified as very good, 10 were good and the remainder poor. Gross oedema, by which is meant fluid in the larger airways was not seen, but slight oedema, recognised as crepitations on palpating the inflated lung, was seen in 8 preparations after an average time of perfusion of 3 hours 10 minutes.

(c) Histological changes

Specimens were taken in 11 experiments (9 consecutive experiments) from the lobes which had appeared best and worst on macroscopical examination. The main pathological findings were similar to those described by Trowell (1943) for dog lungs.

(d) Changes in blood haemoglobin concentration

In the majority of the experiments blood samples were taken for haemoglobin estimation at intervals throughout the perfusion period. Changes in blood haemoglobin concentration were generally negligible for /

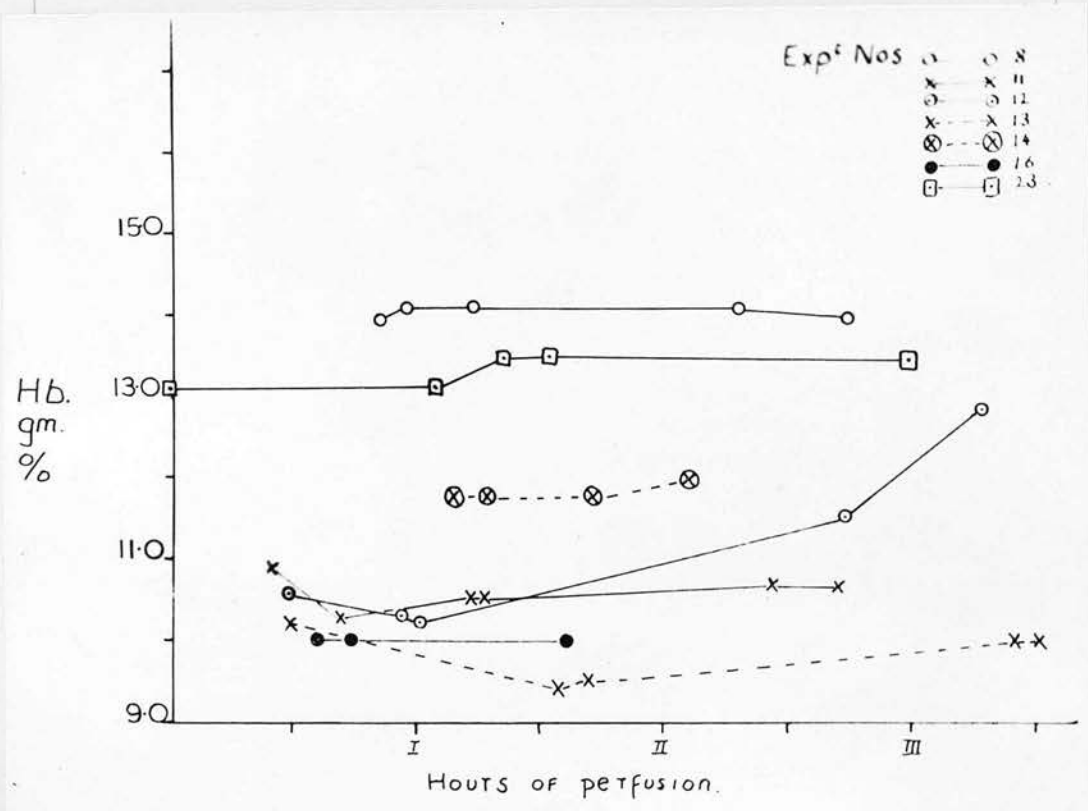


Fig. 2. Changes in blood haemoglobin in relation to the time of perfusion in 7 separate experiments on isolated perfused cat lungs.

for the first 2 to 3 hours of perfusion. Fig. 2 shows the changes in haemoglobin concentration in 7 experiments, the greatest degree of haemoconcentration observed in this series was in experiment 12 in which the blood haemoglobin concentration increased by 20 per cent. in 3 hours perfusion. In the 6 other experiments in Fig. 2 and in 8 experiments not shown the observed changes were less than 5 per cent. of the initial value.

The mechanisms which would account for haemoconcentration in isolated perfused lungs have been fully discussed by Daly et al. (1946). It is not, however, considered that the observed changes in haemoglobin concentration necessarily depict intrapulmonary changes, for instance, the decreased haemoglobin concentration seen in some of the experiments in Fig. 2 might be explained by pooling of red blood corpuscles in the lung or by sedimentation of blood in the apparatus. However, the absence of any marked tendency towards progressive increase of blood haemoglobin concentration is probably additional evidence of the absence of gross oedema in the preparations.

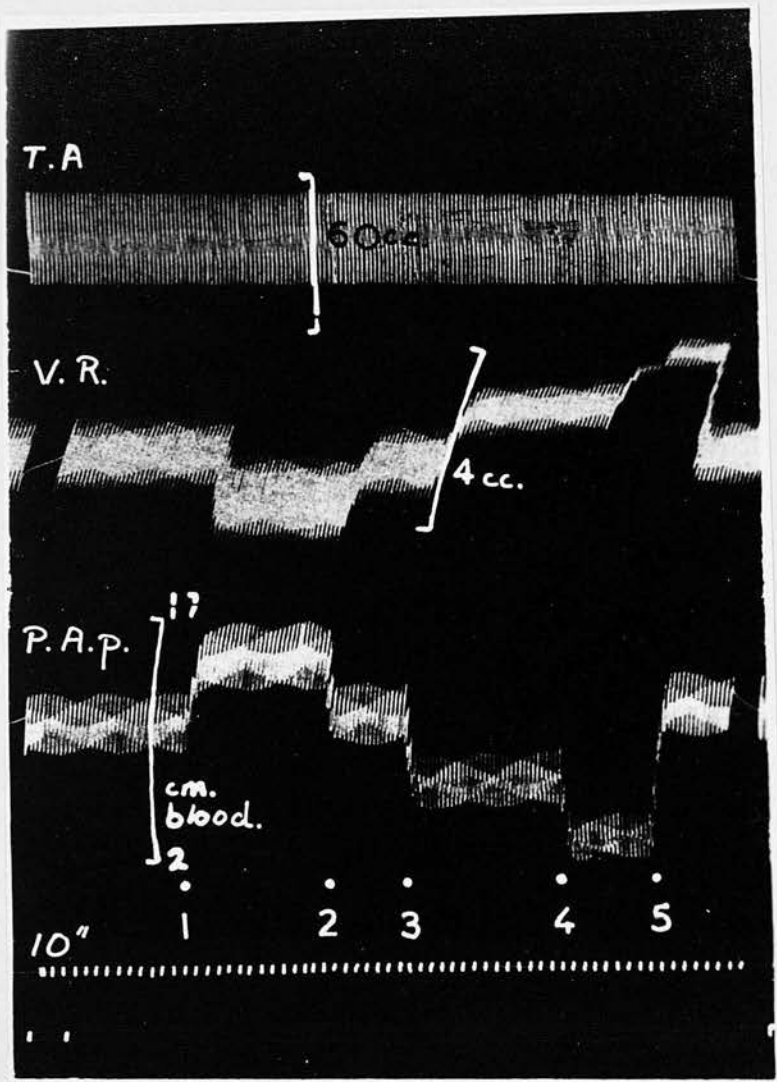


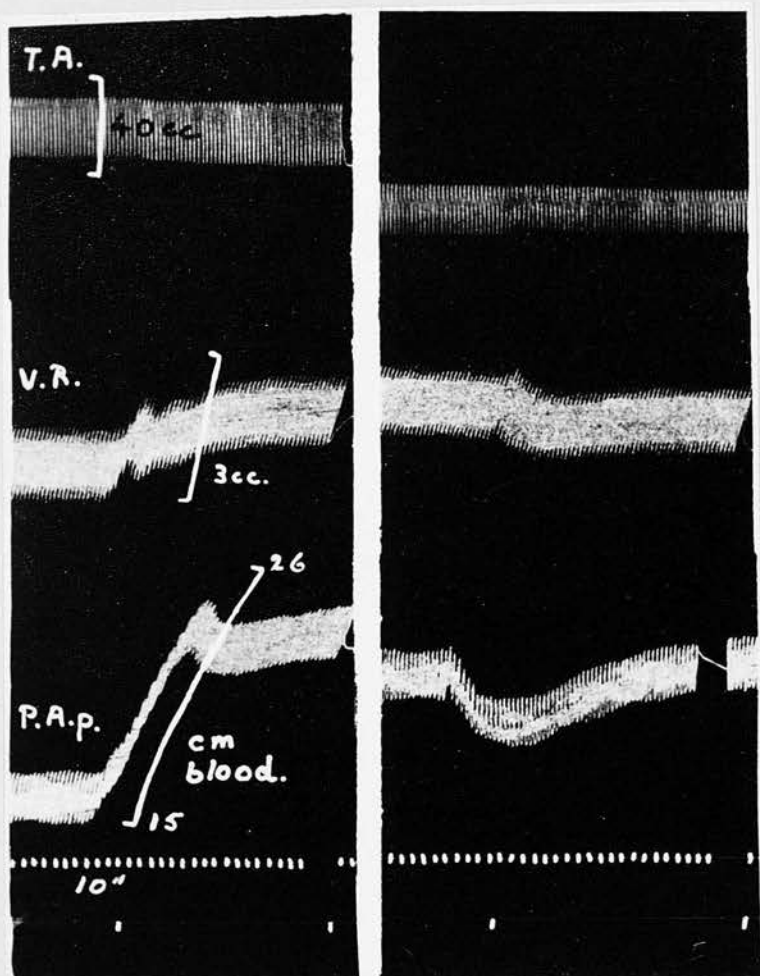
Fig. 3. C.I.P.L. 20. Cat ♂ 3.6 kilos. Chloralose 0.1 gm./kg. body weight given before death of animal. Perfusion begun 10.33 a.m. Negative pressure respiration 0 to -8 cm. H_2O .

11.30 a.m. Effect of variations in blood inflow volume.

Output of pump changed from

1. 135 c.c./min. to 170 c.c./min.
2. 170 c.c./min. to 135 c.c./min.
3. 135 c.c./min. to 100 c.c./min.
4. 100 c.c./min. to 65 c.c./min.
5. 65 c.c./min. to 135 c.c./min.

P.A.p. = pulmonary arterial pressure. V.R. = venous reservoir.



a.

b.

Fig. 4. Same experiment as fig. 3.
 a. 12:03 p.m. 10 μ g. adrenaline injected into the pulmonary artery at signal.
 b. 12:26 p.m. 10 μ g. acetylcholine.

4. Some General Observations on Cat Lungs

(a) The effects of variations in the blood inflow volume

With constant negative pressure ventilation an increase in the minute volume of blood flowing into the pulmonary artery produced an increase of pulmonary arterial pressure and an increase of lung blood volume (see Fig. 3).

(b) The effects of variations in extrapulmonary negative pressure

With constant volume blood inflow an increase in the extrapulmonary negative pressure produced an increase of tidal air and an increase of lung blood volume, the pulmonary arterial pressure was decreased.

(c) Adrenaline

Various doses of adrenaline (Parke Davis' solution with chloretone) were injected into the pulmonary artery in 14 experiments. Initial depressor responses to doses of 10 to 100 μg . were seen in three experiments, in the other experiments doses of 0.1 to 100 μg . caused a pressor response (see Fig. 4). The pressor responses were associated with a slight decrease of lung blood volume, but with the depressor responses the volume of blood in the lungs was slightly increased or unchanged. The larger doses had a prolonged effect on the pulmonary blood vessels, the smaller /

smaller doses were more transient in their effects. Pressor responses to adrenaline were reversed by dihydroergotamine (Sandoz, dose: 0.2 mg. injected into the pulmonary artery).

Gaddum and Holtz (1933) reported a larger number of depressor responses to adrenaline in cat lungs, under somewhat different conditions of test.

Comment

It was decided on the basis of these preliminary observations and tests that the cat isolated perfused lung preparation was a suitable one for experimental purposes, and it appeared to compare favourably with dog lungs as regards the length of time it could be perfused before the vessels lost their reactivity or until oedema ensued. Part of the reason for the success of the experiments may be in the technique of handling the lungs, a technique which was evolved by many workers in this laboratory and has been used with success for the lungs of other species. The lungs were removed from the body and the perfusion begun as rapidly as possible (in practice this time was 20 to 30 minutes). During perfusion the extra-pulmonary negative pressure was kept as low as possible, the visceral pleura was kept moist and excessive heat either in the chamber or of the blood was avoided.

? seconds

It /

It is possible that some or all of three other factors may be of importance in avoiding detriment to the lungs during perfusion.

(1) The use of heparinised blood.- No other work is available on the condition of cat's lungs during perfusion with heparinised blood of homologous origin. Nisell (1948) perfused cats' lungs with diluted heparinised blood from oxen and noted that oedema was common after 4 to 5 hours. Defibrinated blood has been shown to have a strong constrictor action on the pulmonary vessels and bronchi Lühr, (1924) and also on vessels of other regions. Landis, Guerrant and Wood (1943) showed that heparinised blood was without this vasoconstrictor effect on the rabbit's ear. How far the toxic effect of defibrinated blood is responsible for deleterious effects on isolated lungs and what is the mechanism of its prevention by heparin remains to be discovered. It is possible that the trauma attached to the process of defibrination releases a toxic substance from the cells, this is suggested by the results of Landis et al., who found that defibrinated blood still had a constrictor effect after the addition of heparin. In two experiments of this series 10 c.c. of defibrinated blood were injected into the circuit during perfusion with heparinised blood but there was no effect on the blood vessels or bronchi.

(2) Mechanical factors.- From previous work it appears that the lungs in the cat heart-lung preparations are peculiarly susceptible to oedema. In such a preparation it is extremely difficult to avoid a certain degree of venous obstruction during every stage of the dissection. Modrakovski (1914) showed that oedema did not occur easily in cats' lungs unless the venous pressure was increased so that at a pulmonary arterial pressure of 35 mm. Hg., the pressure gradient in the pulmonary circuit was only 8 mm. Hg. In the experiments of the present series the left auricular pressure never exceeded 1 cm. blood and was often lower, and the pulmonary arterial pressure gradient through the lungs was well beyond these limits.

(3) Cleaning of apparatus.- After each experiment all the apparatus was dismantled and washed. All glassware was then soaked in chromic acid for 8 to 12 hours and all the rubber connections were boiled in 5 per cent. sodium carbonate solution. The parts were rinsed many times in distilled water and dried in an incubator. This procedure was adopted because it has been found successful in dogs' lungs perfusions (Daly, personal communication). It is possible that thorough cleaning of all perfusion apparatus is a necessary precaution in preventing the formation of toxic substances.

TABLE I

Effects on the Pulmonary Blood-vessels of Changing the Ventilating Gas Mixtures from Air to Air containing 5 per cent. CO₂

No. of Expt.	Weight of dog, kg.	Time from start of perfusion to change	Duration of administration of 5 per cent. CO ₂	I.B.V. diminution c.c.	Maximum P.A.p. change cm. blood
*2	17 (1 hr. 12 min.	50 min.	16	+ 2.5
	(2 hr. 54 min.	1 hr. 13 min.	7.5	+ 3.0
*3	12	3 hr. 38 min.	44 min.	31	+ 2.3
6	12.8	1 hr. 45 min.	3 min.	13	+ 1.0
*13	10.3	3 hr. 25 min.	42 min.	32	+ 1.0
14	8	55 min.	36 min.	21	None
15	12.8	2 min.	48 min.	27	- 1.5
16	13.8	57 min.	42 min.	24	- 1.5
17	13.0	53 min.	41 min.	18.5	- 0.5
18	9.0	57 min.	29 min.	23	None
		58 min.	21 min.	19	+ 0.75
		18 min.	21 min.	9	+ 0.5
20	21.0 (3 hr. 45 min.	21 min.	9	+ 0.5

*Positive pressure ventilation

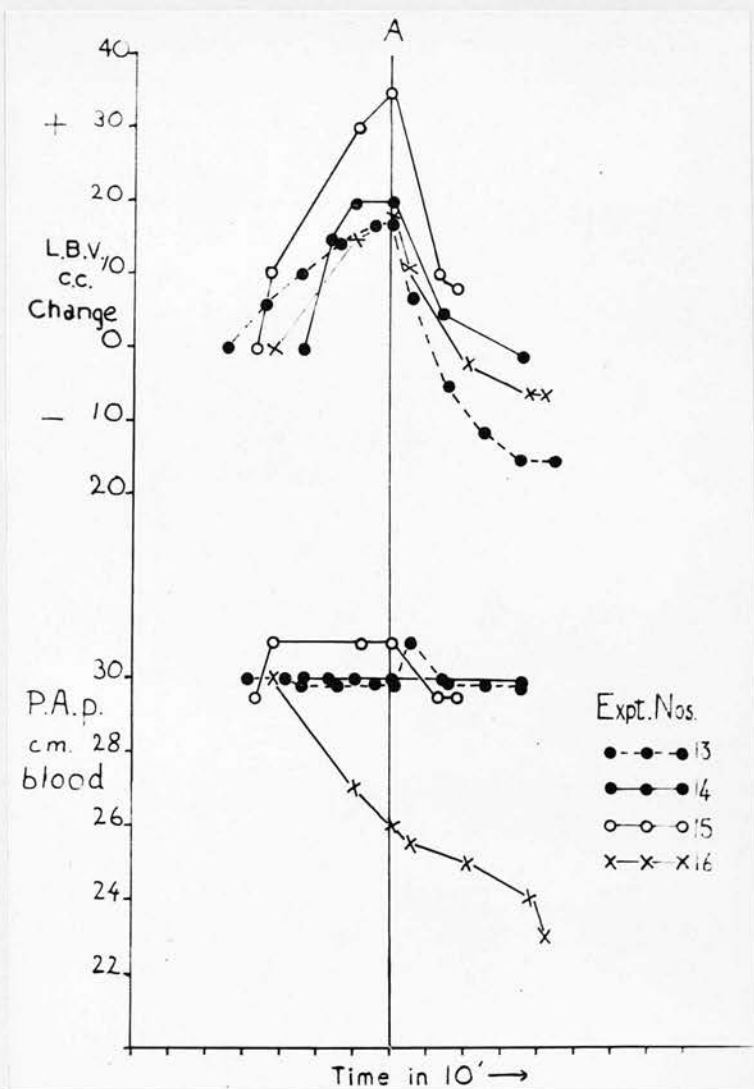
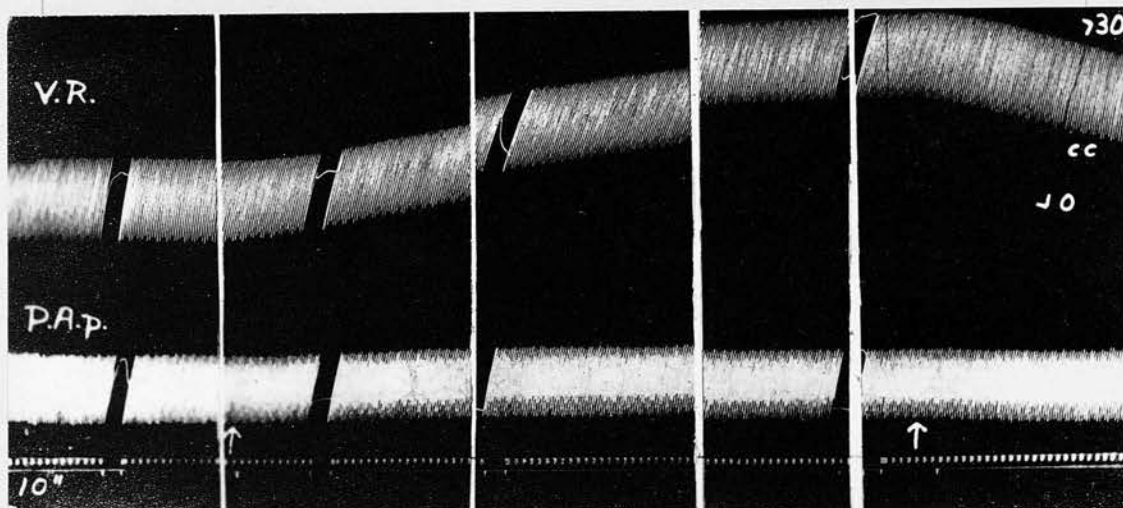


Fig. 5. The effect of changing the ventilating gas mixture from air to air containing 5% CO_2 . The initial L.B.V. level is arbitrarily taken as zero. The vertical line (A) marks the time of the change of gas mixture in each experiment. Positive pressure ventilation was used in experiment No. 13. In Nos. 14, 15 and 16 the lungs were ventilated by negative pressure.

L.B.V. = lung blood-volume.



a. b. c. d. e.

Fig. 6. Expt. 14. Dog, 8.0 kg. Chloralose (0.1 g./kg.) given before bleeding. Negative pressure ventilation. Perfusion begun 11.45 a.m.

a. 12.35 p.m. Ventilation with air.

b. 12.37 p.m. Arrow and signal mark time of change to 5% CO₂ in air. The increased volume of blood in the V.R. shows a decrease of L.B.V.

c. 12.47 p.m.

d. 1.06 p.m.

e. 1.16 p.m. Change to air at signal and arrow. No change in P.A.p. which remained at 30 cm. blood.

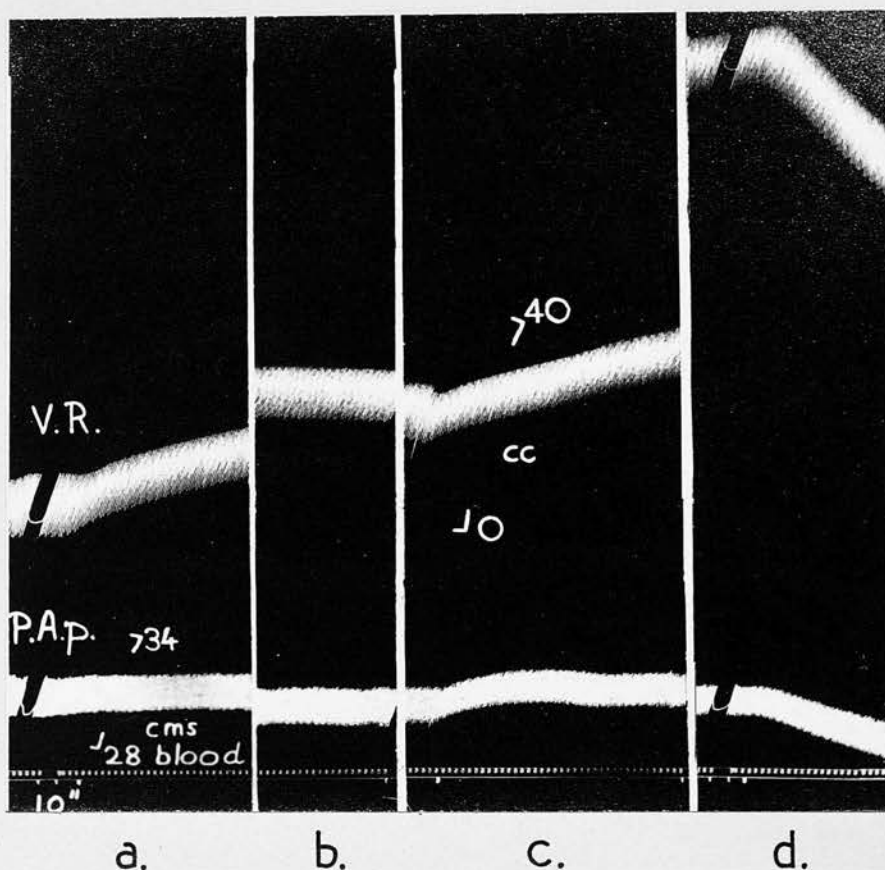


Fig. 7. Expt. 15. Dog, 12.8 kg.
Negative pressure ventilation. Perfusion
begun at 11.30 a.m.

- a. 12.32 p.m. Change from air to air
containing 5% CO_2 at signal. Tidal air
252 c.c.
- b. 12.56 p.m. Tidal air 215 c.c.
- c. 1.24 p.m. Change from air containing
5% CO_2 to air containing 10% CO_2 (at
signal). Tidal air 200 c.c.
- d. 1.55 p.m. Change from air containing
10% CO_2 to air (at signal). Tidal air
200 c.c.

TABLE II

Effects on the Pulmonary Blood-vessels of Changing the Ventilating Gas Mixture from Air containing 5 per cent. CO₂ to Air containing 10 per cent. CO₂

No. of Expt.	Weight of dog, kg.	Time from start of perfusion to change	Duration of administration of 10 per cent. CO ₂	L.B.V. diminution c.c.	Maximum P.A.p. change cm. blood
*13	10.3	4 hr. 7 min.	min. 18	19	+ 0.5
15	12.8	1 hr. 50 min.	31	65	+ 2.25
16	13.8	1 hr. 39 min.	23	30	- 1.5
17	13.0	1 hr. 34 min.	14	27.5	+ 0.5
18	9.0	1 hr. 26 min.	25	15	None

*Positive pressure ventilation

IV. RESULTS

1. The Effects of Carbon Dioxide on Dog Lungs

A change in the ventilating gas mixture from air to air containing 5 per cent. CO_2 (42 tests in 24 experiments) was followed, after a latent period of 10 to 30 seconds by a decrease in lung blood volume. The loss of blood from the lungs was at first rapid, and then gradually reached an asymptote after a period which varied from 16 to 46 minutes, although the administration of 5 per cent. CO_2 was usually continued for a longer period. The total diminution of lung blood volume was of the order of 20 to 30 c.c. although some preparations were less sensitive (see Table I, experiments 2 and 6). Fig. 5 shows the effect of 5 per cent. CO_2 on the pulmonary arterial pressure and lung blood volume in four experiments and Fig. 6 is a tracing obtained from one of the experiments shown in Fig. 5. Similar results were obtained when the control respiratory mixture was oxygen and the test mixture 5 per cent. CO_2 in O_2 . In 5 experiments the gas mixture was changed from air containing 5 per cent. CO_2 to air containing 10 per cent. CO_2 and a further diminution of lung blood volume, which varied from 15 to 65 c.c., then occurred (see Table II and Fig. 7). These values do not represent the /

the total diminution of lung blood volume which could have been produced by 10 per cent. CO_2 , since the lung blood volume was still falling in every test when ventilation with 10 per cent. CO_2 was stopped. With pure CO_2 , which was administered for only a short period (1 to 3 minutes) in three experiments, the lung blood volume diminished more rapidly than with lower concentration, but the full effects of pure CO_2 were not measured because the period of exposure was purposely restricted.

These results were independent of the use of chloralose anaesthesia in the preparation and of the use of positive or negative pressure ventilation.

In only two experiments out of 26 was CO_2 without effect on the lung blood volume. On one of these occasions there had been an accidental obstruction to the venous outflow for the first hour of perfusion and the lungs were rapidly becoming oedematous. On the other occasion the extrapulmonary negative pressure had been increased much beyond that normally used and the lungs were rapidly taking up blood.

Changes in pulmonary arterial pressure were not so consistently observed as were changes in lung blood volume. A slight initial increase of pulmonary arterial pressure, which was seldom maintained during the whole period of administration of the gas mixture containing /

containing excess CO_2 , was observed in approximately 50 per cent. of the tests. Fig. 11 shows an increase of pulmonary arterial pressure following ventilation of the lungs with 5 per cent. CO_2 in air (from air). In another experiment the pulmonary arterial pressure subsequently fell to below its original level 10 minutes after the change in gas mixture without any effect on the rate of diminution of lung blood volume. An increase of pulmonary arterial pressure in response to 5 per cent. CO_2 was seen in all the experiments in which positive pressure ventilation was used, and in these the tidal air was approximately 20 to 30 c.c./kg. body-weight. Of 16 tests made in 9 consecutive experiments in which negative pressure ventilation was used, 5 pressor responses were obtained. The tidal air was greater than 13 c.c./kg. body-weight in each of these, but was less than 13 c.c./kg. body-weight in 9 out of the other 11 tests. Applying the χ^2 test (Bradford Hill, 1945) $\chi^2 = 6.32$ and p is less than 0.01. Thus the presence or absence of a pulmonary pressor response may be related to the volume of the tidal air. The concentration of CO_2 inhaled also determined to some extent whether or not a pressor response was observed. Thus in Fig. 7 no increase of pulmonary arterial pressure followed the administration of 5 per cent. CO_2 , but increasing the concentration /

concentration of CO_2 to 10 per cent. produced a rise of pulmonary arterial pressure.

These observations suggest that the increase in pulmonary arterial pressure which was sometimes observed was related to the rate of increase or the alveolar concentration of CO_2 . This was also suggested by Hebb and Nimmo Smith (1947) as a reason for the difference between their results on dog and Macacus rhesus lungs.

In these experiments very large changes in blood CO_2 content were produced by inhalation of air containing 5 or 10 per cent. CO_2 instead of atmospheric air. The CO_2 content of the pulmonary arterial blood was often as low as 3.0 vol. per cent. after the lungs had been ventilated with air for approximately one hour. While 40 to 50 minutes after changing to 5 per cent. CO_2 in air it was of the order of 30 vol. per cent. and ventilation with 10 per cent. CO_2 for 15 to 30 minutes increased it still further to at least 50 vol. per cent. The oxygen content of the arterial blood remained nearly constant in each experiment (with variations of less than 1 vol. per cent.), and varied between 16 and 20 vol. per cent. in different experiments. It was not possible to calculate the volume of blood in the lungs at the beginning of perfusion, and for this reason it is not possible to express the changes in lung blood volume as /

TABLE III

No. of Expt.	Time from start of perfusion to test	Lung blood-volume change c.c. from an arbitrary initial zero	Blood CO ₂ content volume per cent. at time of test
6	0-1 hr. 45 min. 2 hr. 48 min.	+ 35 - 13	3.82 33.01
13	3 hr. 38 min. 2 hr. 36 min. - 3 hr. 25 min. 4 hr. 7 min.	+ 68 + 15 - 32	3.42 16-4.44 28.93
15	0-1 hr. 2 min. 1 hr. 50 min. 2 hr. 21 min.	+ 35 - 27 - 55	8.42 34.76 62.03
16	0-0 hr. 57 min. 1 hr. 2 min. 1 hr. 39 min. 2 hr. 2 min. 2 hr. 29 min.	+ 18 - 7 - 17 - 30 + 49	12.02 27.06 34.62 52.35 21.09
17	0-0 hr. 53 min. 58 min. 1 hr. 34 min. 1 hr. 48 min.	+ 33.5 - 6 - 12.5 - 27.5	8.25 28.33 35.65 51.74

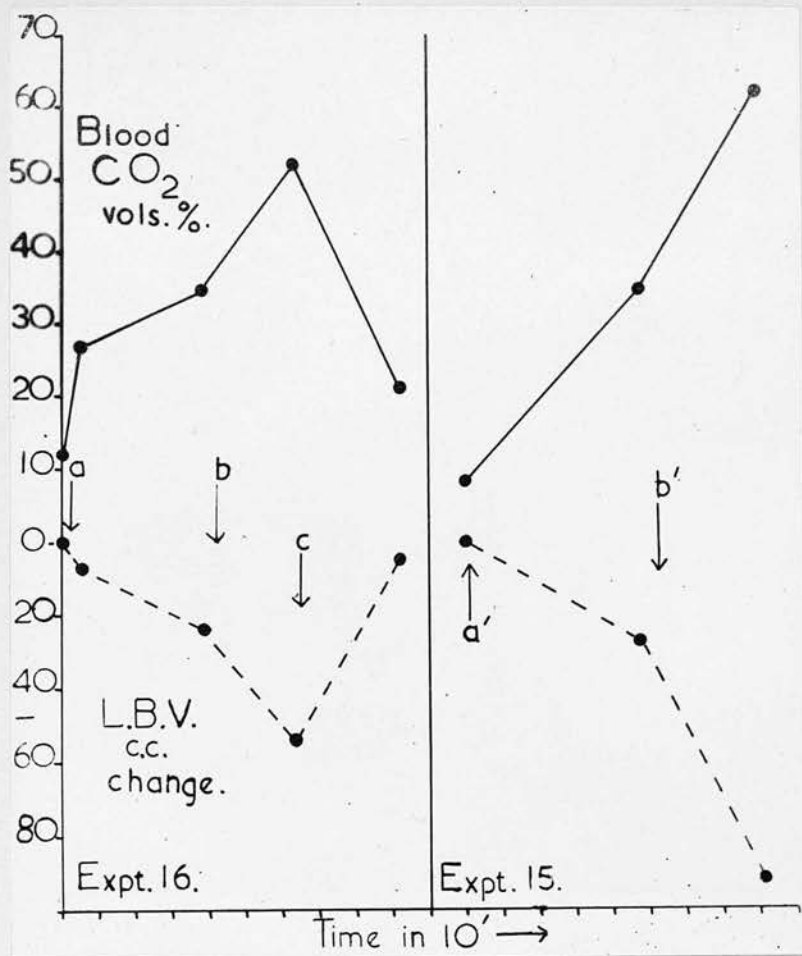


Fig. 9. The inverse relationship between the CO₂ content of the pulmonary arterial blood and the volume of blood in the lungs. The lung blood-volume is plotted from an arbitrary zero. Two experiments are shown, both of which were carried out under negative pressure ventilation. The times of the changes of gas mixtures are shown thus:
Expt. 16.

a. Air to air containing 5% CO₂

b. Air containing 5% CO₂ to air containing 10% CO₂.

c. Air containing 10% CO₂ to air.

Expt. 15.

a. Air to air containing 5% CO₂

b. Air containing 5% CO₂ to air containing 10% CO₂.

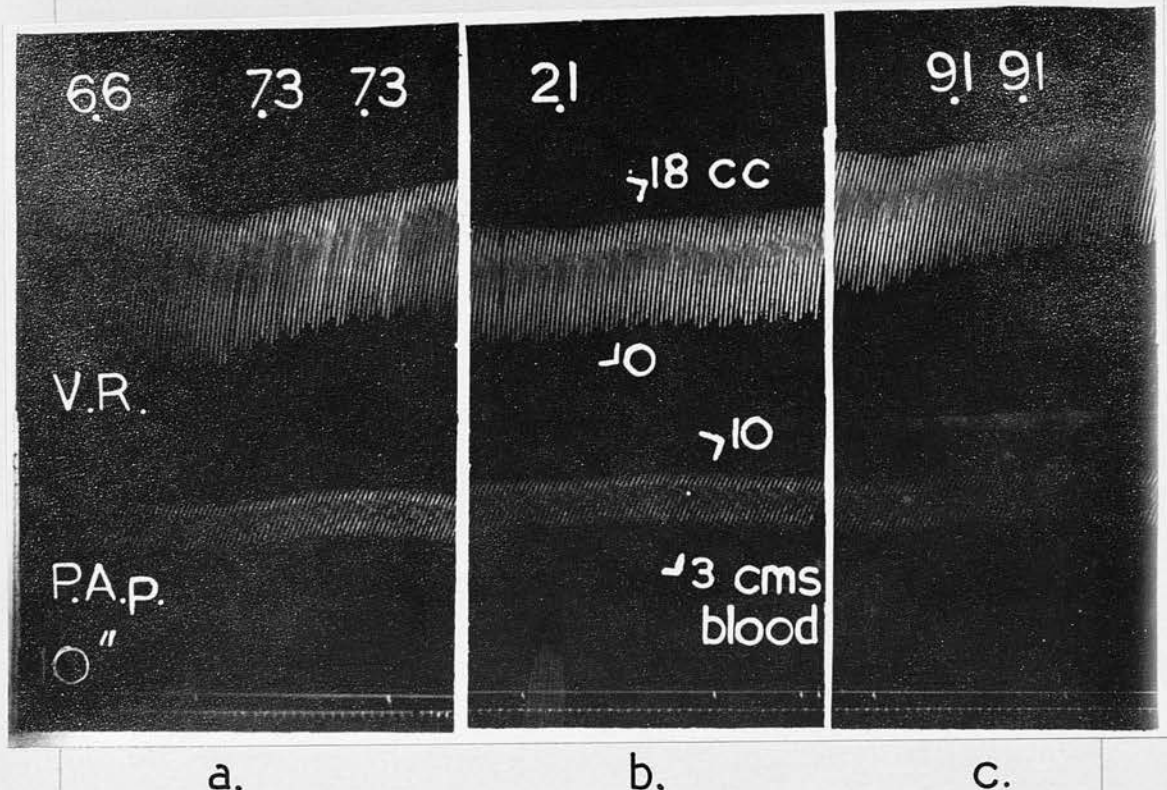


Fig. 10. Expt. 25. Dog, 15.0 kg. Perfusion begun at 11.03 a.m.

a. 1.17 p.m. Negative pressure ventilation.

b. 2.00 p.m.

c. 2.12 p.m.

Ventilation was with air and a change made to 10% CO₂ in air between the signals.

2 mgm. dihydroergotamine given between a and b at 1.47 p.m. (concentration in blood 1:225,000).

The lungs were inflated once by positive pressure between b and c.

The top row of figures is the tidal air in c.c.

as percentage changes of the total volume of blood in the lungs. However, in any one experiment the amount of blood in the lungs relative to an arbitrary initial value was inversely related to the CO_2 content of the arterial blood (see Fig. 9 and Table III).

The response of the pulmonary blood vessels to CO_2 was not suppressed by ergotoxine ethane sulphonate (4 experiments) or by dihydroergotamine (Sandoz) (2 experiments). Both drugs were injected into the pulmonary arterial tubing to give blood concentrations (1:400,000-1:200,000) which were sufficient to reverse or to inhibit the pulmonary pressor response to adrenaline. The CO_2 response was usually reduced after ergotoxine and ergotamine. Since both these drugs diminished the tidal air, it appeared likely that they also diminished the amount of CO_2 admitted to the alveolus, a view which was supported by blood gas analysis. In the experiment of Fig. 10 the CO_2 response, which had been much reduced after dihydroergotamine was again improved when the tidal air was restored by inflating the lungs under positive pressure.

Additions of atropine sulphate to the blood (conc. 1:400,000-1:500,000) did not inhibit or otherwise affect the pulmonary vascular response to CO_2 (3 experiments).

When /

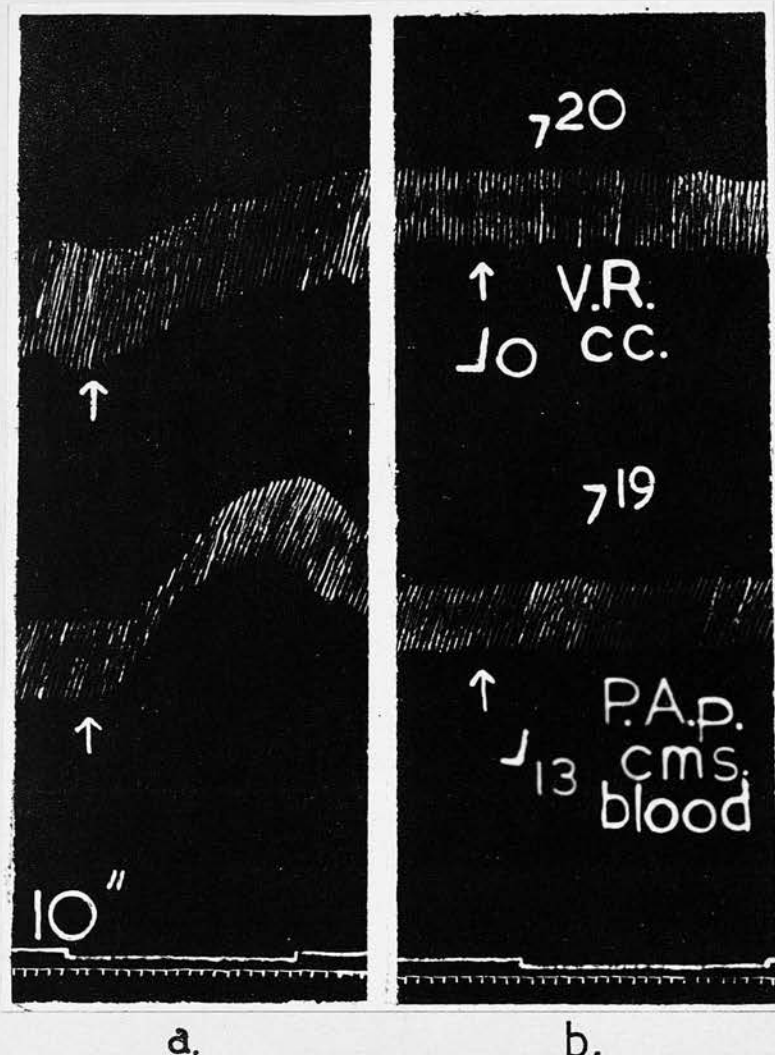


Fig. 8. Expt. 24. Dog, 16.3 kg. Chloralose 0.1 g./kg. given before bleeding. Circulation through lungs stopped for 2 minutes between death of animal and perfusion. Perfusion started at 12.38 p.m.

- a. 3.19 p.m. Change from air to air containing 5% CO₂. Tidal air 450 c.c.
- b. 4.17 p.m. Control test with Douglas bag of air. Negative pressure 0 to -16 cm. H₂O at a. 0 to -10 cm. H₂O at b.

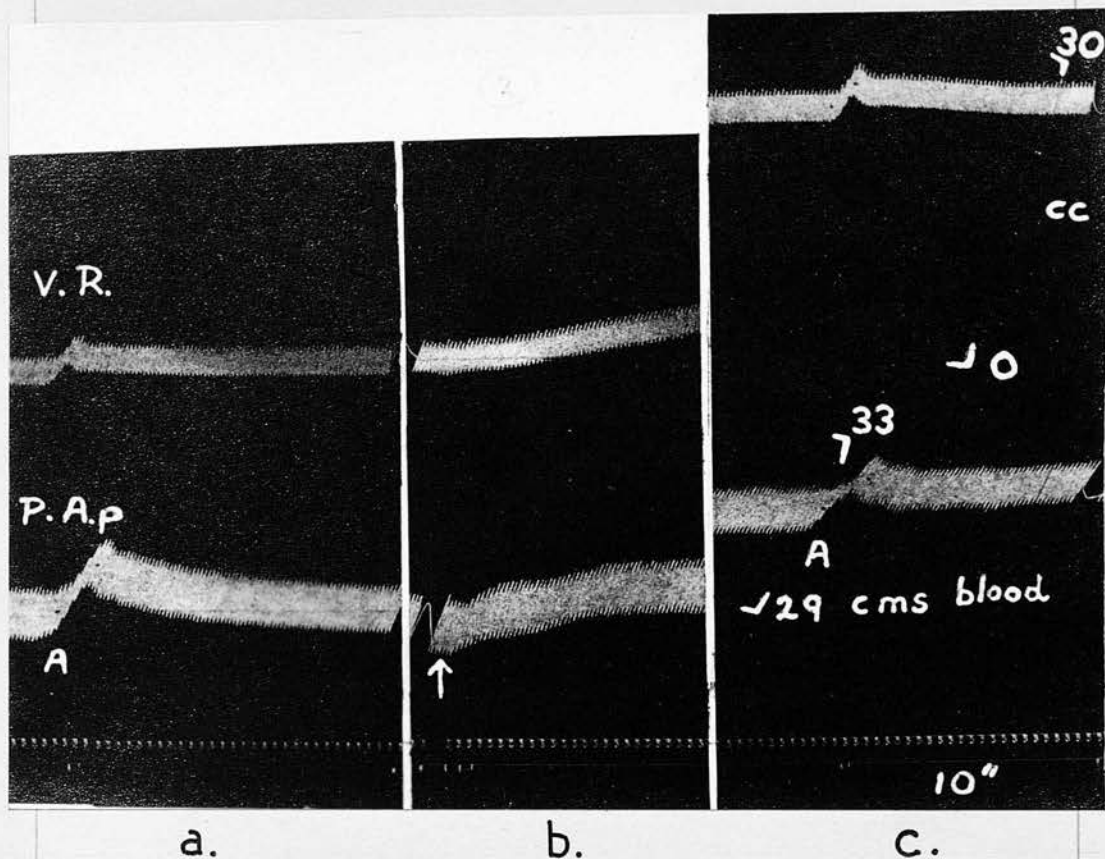


Fig. 11. Expt. 3. Dog, 12.0 kg.
 540 c.c. blood in apparatus. Blood-flow
 310 c.c./min. Perfusion begun at 11.05
 a.m. Positive pressure ventilation.
 10 μ g. adrenaline was injected into the
 pulmonary artery every 10 minutes through-
 out the experiment.

- a. 2.30 p.m. Ventilation on air. 10 μ g.
 adrenaline at A.
- b. 2.43 p.m. Change to air containing
 5% CO_2 at signal and arrow.
- c. 3.20 p.m. 37 minutes after changing
 to air containing 5% CO_2 . 10 μ g.
 adrenaline at A.

When the lungs were ventilated by negative pressure, alterations in the CO_2 concentration of the ventilating gas mixtures did not produce changes in the tidal air; and control tests made with Douglas bags filled with air instead of a test gas mixture gave negative results (see Fig. 8). Thus mechanical sources of error due to changes in bronchial calibre and alterations of intra-alveolar pressure were excluded.

Responses of the pulmonary vessels to adrenaline during ventilation of the lungs with 5 per cent. CO_2 in air

Adrenaline was injected into the pulmonary artery in 13 experiments to see if variations in blood CO_2 content had any effect on the response of the lung blood vessels. The dose of adrenaline was kept constant in each experiment (5 to 20 μg . of Parke Davis' solution with chloretone) and tests were repeated at 10-minute intervals. The response was always the same - there was a transient rise in pulmonary arterial pressure and a coincident increase in the venous outflow. There was no difference in the response when air or air containing 5 per cent. CO_2 was used to ventilate the lungs, as shown in Fig. 11 in which 10 μg . adrenaline was injected into the pulmonary artery before and during administration of /

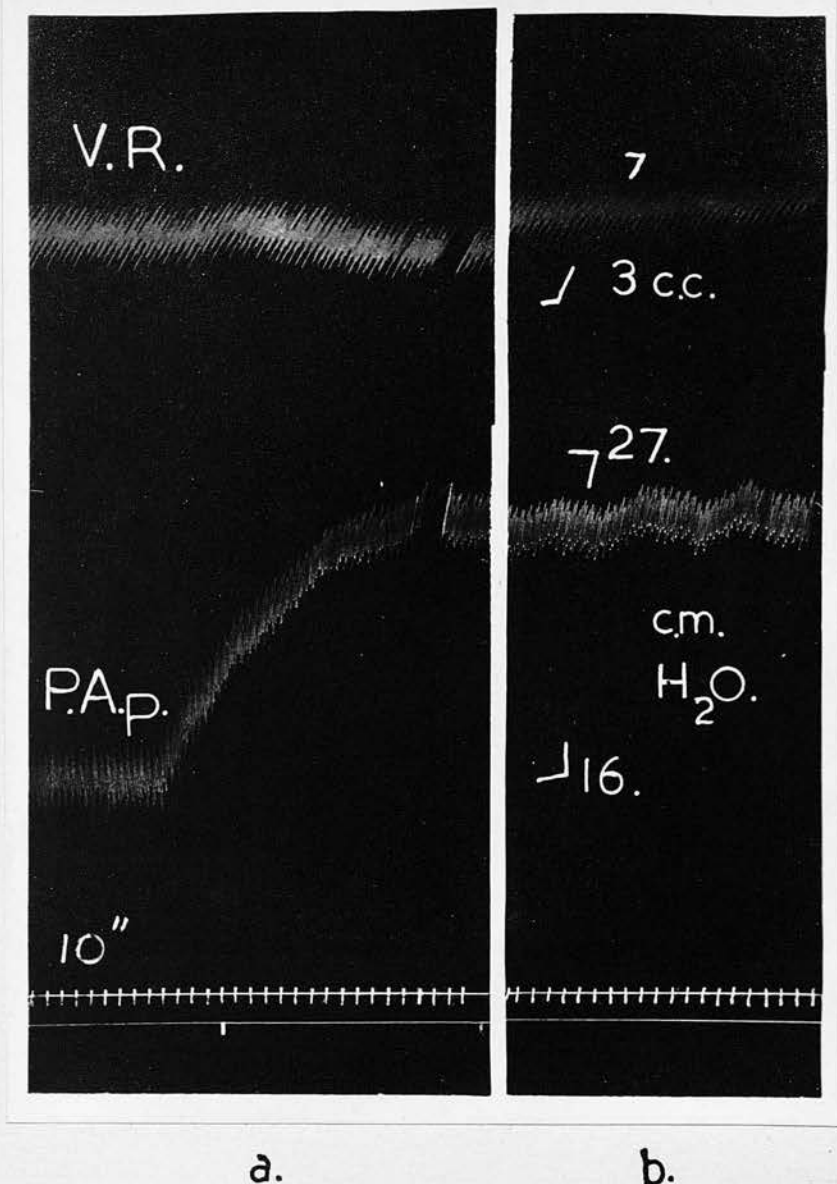


Fig. 12. C.I.P.L. 12. Cat ♂ 3.17 kg.
 Perfusion begun at 11.15 a.m. Negative pressure respiration 0 to -10 cm. blood.

a. 2.53 p.m. Ventilating gas mixture changed from air to 10% CO₂ in air.

b. 2.59 p.m. Still respiring 10% CO₂ in air. Pulmonary arterial pressure has maintained a new level. Slight increase of blood in venous reservoir.

of 5 per cent. CO_2 in air. Additions of adrenaline to the blood did not vary the usual pulmonary vascular response to 5 per cent. CO_2 , although Löhner (1924) obtained a reversal of the CO_2 response in cat lungs after much larger doses of adrenaline.

2. The Effects of CO_2 on Cat Lungs

A change in the ventilating gas mixture from air to air containing 5 per cent. or 10 per cent. CO_2 produced a pulmonary pressor response (see Table IV). The increase of pulmonary arterial pressure was visible after a latent period of 10 to 30 seconds, and had reached a maximum within three minutes, thereafter it often fell slightly, although the excess CO_2 was still inhaled. The maximum pressure change observed with 10 per cent. CO_2 was an increase of 96 per cent. above the initial level, more usually, however, the increase was only 10 to 30 per cent. Fig. 12 shows the response to 10 per cent. CO_2 obtained in one experiment. There was no apparent correlation between the duration of the administration of the CO_2 mixture or the rate of increase of CO_2 content of the arterial blood and the intensity of the pressor response. Therefore the variations in the response must be attributed to variations in sensitivity between the preparations.

The /

TABLE IV

The Effects of Carbon Dioxide on Cat Lungs

No. of expt.	Anaesthetic	Weight of Cat (kg.)	Time from Start of Perfusion to Test	Duration of Administration of 10% CO ₂ (minutes)	P.A.p. Change		L.B.V. Change (c.c.)	% Sat. Art. Blood		Blood O ₂ vols. %		Blood CO ₂ vols %	
					Actual cm. blood	% increase		Before	After	Before	After	Before	After
(a) Change from Air to Air containing 10% CO ₂													
7	N	2.6	3 hr. 7 min.	5	26.6-44.7	96	Nil	-	-	16.77	16.29	5.44	17.29
8	N	3.4	2 hr. 38 min.	6	27.5-35.9	30.6	Nil	96	87	16.87	16.60	2.76	22.62
11	C	2.6	38 min.	10.5	17.5-18.5	5.7	-1.5	97.5	89.7	14.35	13.24	3.20	29.68
(1 hr. 58 min.	17	22.0-24.1	9.6	-2.0	-	-	-	-	-	-
(1 hr. 39 min.	6	12.4-14.0	12.9	Nil	86.8	81.0	11.31	10.79	4.30	23.33
16	C	3.5	2 hr. 9 min.	6	12.5-13.25	6.0	Nil	-	-	-	-	-	-
(2 hr. 22 min.	6	13.1-14.4	11.0	Nil	-	-	-	-	-	-
(2 hr. 35 min.	6	12.3-13.0	6.0	Nil	-	-	-	-	-	-
17	C	2.8	1 hr. 8 min.	10	16.4-17.3	6.0	-2.0	92.3	87.0	14.16	13.36	4.54	33.07
6	N	4.1	1 hr. 18 min.	10.5	21.9-24.0	10.0	Nil	-	-	-	-	-	-
10	C	3.1	1 hr. 4 min.	13	19.9-27.5	38.0	Nil	-	-	-	-	-	-
13	C	3.2	3 hr. 38 min.	14	16.4-26.0	58.5	-2.0	-	-	-	-	-	-
(b) Change from Air to Air containing 5% CO ₂													
3	N	2.5	3 hr. 21 min.	14	28.0-34.0	21.0	-2.75	-	-	-	-	-	-
(4 hr. 50 min.	18	26.0-32.0	23.0	-1.25	-	-	-	-	-	-

C = Chloralose.

N = Nembutal

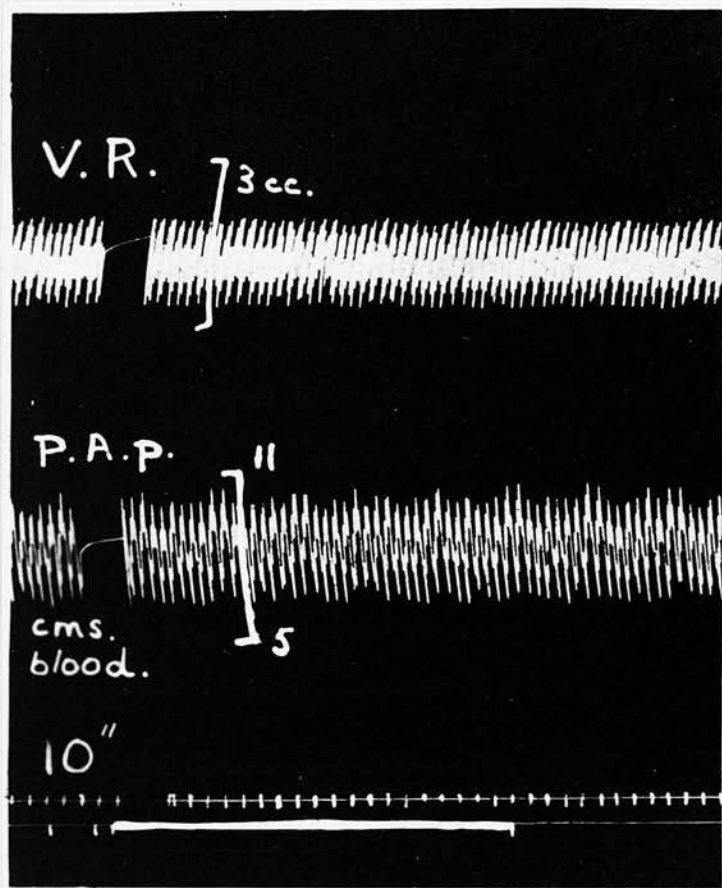


Fig. 13. C.I.P.L. 16. Cat ♂ 3.5 kg. Chloralose anaesthesia. Perfusion begun 11.00 a.m. Negative pressure respiration -1 to -10 cm. H_2O . Control test of the method of administration of gas mixtures.

2.30 p.m. A Douglas bag of air was given during the period indicated by the signal.

The intensity of the response did not appear to increase with increasing time from the beginning of perfusion (see experiment 15 Table IV)

CO₂ did not produce marked changes in lung blood volume in cats' lungs. A slight diminution of 1 to 2 c.c. occurred in 1/3 of the tests with 10 per cent. CO₂ but in the other tests no such change was observed.

The results were not influenced by the use of positive or negative pressure ventilation or by the use of chloralose or nembutal anaesthesia (see Table IV).

Ventilation of the lungs with air containing 5 or 10 per cent. CO₂ did not produce marked changes in tidal air in these preparations, although slight changes in tidal air might not have been demonstrable with the apparatus and technique used. It is, however, unlikely that the effects could have been caused by changes in bronchial calibre. Mechanical alterations in intrapulmonary pressure might produce alterations in pulmonary arterial pressure, but these were also excluded by negative control tests of the method of administration of the gas mixtures (see Fig. 13).

The pulmonary arterial pressor response to CO₂ was not suppressed or reversed by the addition of dihydroergotamine (Sandoz) to give a final concentration (1:5,000) which was sufficient to reverse or suppress /

TABLE V

The Effects of Oxygen on Cat Lungs

No. of Expt.	Anaesthetic	Weight of Cat (kg.)	Time from Start of Perfusion to Test	Duration of Administration of O ₂ (minutes)	P.A.p. Change		L.B.V. Change (c.c.)	% Sat. Art. Blood		Blood O ₂ vols. %		Blood CO ₂ vols. %	
					Actual cm. blood	% increase		Before	After	Before	After	Before	After
(a) Change from Air to O ₂													
3	N	2.5	52 min.	16	19.5-21.0	+2.4	Nil	-	-	-	-	-	-
6	N	4.1	52 min.	3	Nil	-	Nil	-	-	-	-	-	-
7	N	2.6	1 hr. 20 min.	5	27.0-26.9	-0.4	Nil	-	-	15.82	15.97	9.3	8.48
8	N	3.4	1 hr. 52 min.	3	24.75-24.9	+0.6	Nil	-	-	16.26	18.2	5.84	6.8
11	C	2.6	52 min.	5	Nil	-	Nil	96.0	96.0	17.76	17.76	3.77	3.48
			21 min.	10	24.1-24.6	+2.1	Nil	91.0	99.0	17.0	17.64	-	-
12	C	3.2	2 hr. 8 min.	10	Nil	-	Nil	-	-	-	-	-	-
12	C	3.2	26 min.	5	19.0-18.1	-4.7	-0.1	87.0	99.0	13.75	14.07	9.0	8.24
			20 min.	5	11.5-12.3	+7.0	Nil	97.0	100.0	16.6	17.9	1.13	1.55
(b) Change from Air + 10% CO ₂ to O ₂ + 10% CO ₂													
9	C	4.3	1 hr. 12 min.	5	31.7-31.1	-1.9	Nil	-	-	-	-	-	-
10	C	3.1	47 min.	4	15.1-14.9	-1.3	Nil	-	-	16.50	15.84	49.59	41.28
			20 min.	6	16.1-14.9	-7.5	Nil	-	-	14.25	14.50	44.40	42.37
11	C	2.6	2 hr. 15 min.	5	Nil	-	Nil	-	-	-	-	-	-
11	C	2.6	38 min.	13	19.0-18.1	-4.7	Nil	95.7	100.0	13.39	14.28	31.40	31.75
			16 min.	5	19.1-18.4	-3.7	Nil	-	-	-	-	-	-
12	C	3.2	3 hr.	10	Nil	-	Nil	-	-	-	-	-	-
12	C	3.2	31 min.	5	12.27-11.8	-3.8	Nil	-	-	15.09	17.76	38.4	38.21
			51 min.	5	Nil	-	Nil	-	-	15.16	16.83	38.6	35.28
13	C	3.2	1 hr. 23 min.	5	15.2-15.0	-1.3	Nil	-	-	-	-	-	-
13	C	3.2	31 min.	5	18.6-18.25	-0.2	Nil	-	-	13.95	14.89	50.66	50.32
			6 min.	5	22.4-21.6	-0.4	Nil	-	-	13.86	14.72	50.78	45.73
14	C	2.7	2 hr. 25 min.	5	19.8-18.4	-7.1	Nil	-	-	-	-	-	-
			1 hr. 12 min.	5	Nil	-	Nil	-	-	-	-	-	-

C = Chloralose.

N = Nembutal

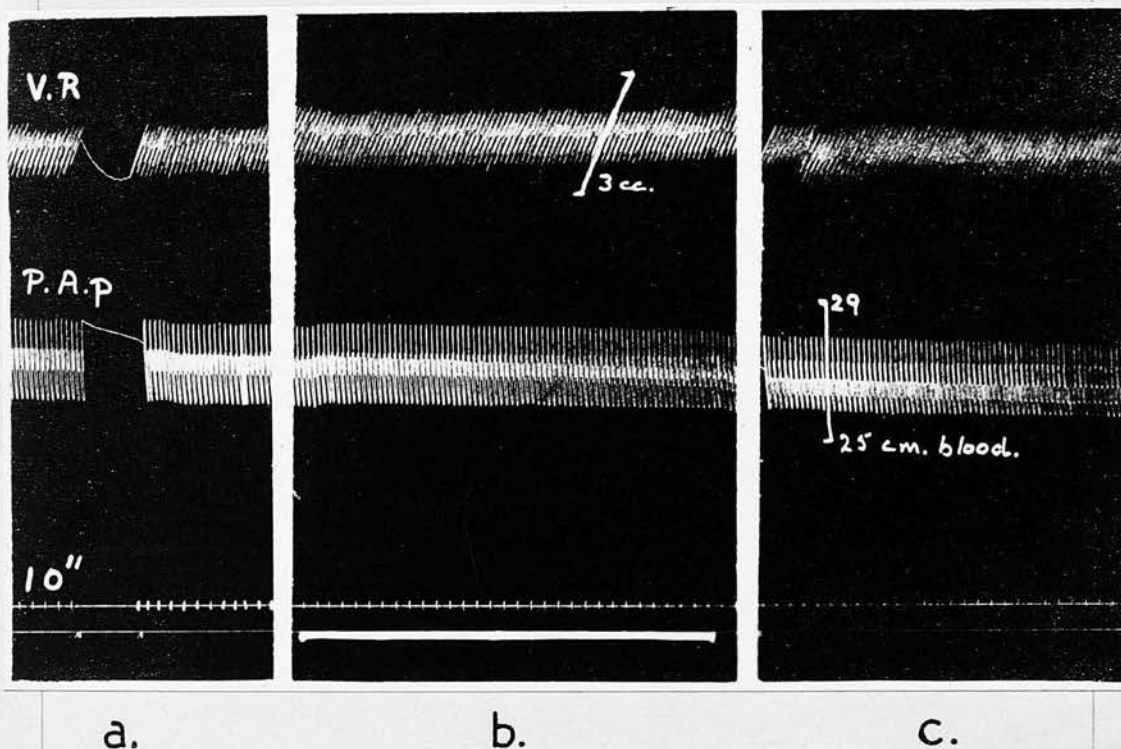


Fig. 14. C.I.P.L. 7. Cat ϕ 2.6 kg. Nembutal anaesthesia. Perfusion begun 11.30 a.m. Negative pressure respiration 0 to -10 cm. H_2O .

a. 12.47 p.m. Ventilation with air.

b. 12.50 p.m. O_2 given between signals on bottom line.

c. 12.57 p.m. AIR.

Blood sample taken between

a and b CO_2 9.3 vol.% O_2 15.82 vol.%
b and c CO_2 8.48 vol.% O_2 15.97 vol.%

suppress the adrenaline response. Atropine sulphate (conc. 1:500) also did not affect the response.

3. The Effects of O₂ on Cat Lungs

The effects of the inhalation of pure O₂ (instead of air) have been observed in 6 experiments (9 tests). This change in gas mixture produced no variation in lung blood volume and had a slight and inconsistent effect on the pulmonary arterial pressure (see Table Va and Fig. 14). The oxygen content and percentage saturation of the arterial blood was however increased in every test in which the information is available (with one exception).

Table Vb shows that if the CO₂ content of the gas mixture was kept constant at 10 per cent., then a change from air containing 10 per cent. CO₂ to O₂ containing 10 per cent. CO₂ had similarly no marked effect on the pulmonary arterial pressure or lung blood volume. The slight fall of pressure which occurred in these tests was not more marked than could be accounted for by the slight decrease in blood CO₂ content which also occurred.

Those negative results were obtained in experiments using positive or negative pressure ventilation and with chloralose or nembutal anaesthesia.

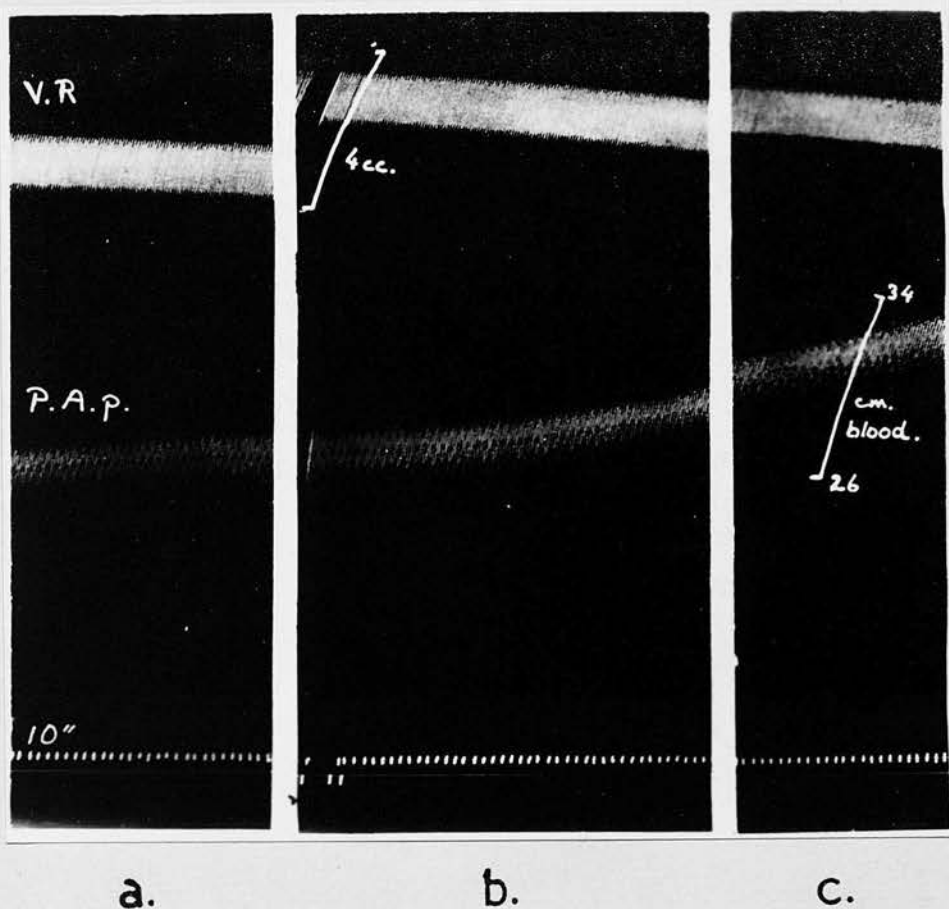


Fig. 15. C.I.P.L. 25. Cat ♂ 3.8 kg. Chloralose anaesthesia. Perfusion begun 11.25 a.m. Negative pressure ventilation 0 to -10 cm. H_2O .

a. 1.18 p.m. Ventilation with air.

b. 1.25 p.m. Change to N_2 at signal.

c. 1.35 p.m. Ventilation still on N_2 .

Height of volume recorder adjusted between a and b, and b and c.

Blood samples.

1.24 p.m. CO_2 18.20 vol.% O_2 13.55 vol.%

1.34 p.m. CO_2 18.14 vol.% O_2 12.91 vol.%

1.40 p.m. CO_2 18.23 vol.% O_2 13.06 vol.%

TABLE VI

The Effects of Nitrogen on Cat Lungs

No. of Expt.	Anaesthetic	Weight of Cat (kg.)	Time from Start of Perfusion to Test	Duration of Administration of N ₂ (minutes)	P.A.p. Change		L.B.V. Change (c.c.)	% Sat. Art. Blood		Blood O ₂ vols. %		Blood CO ₂ vols. %	
					Actual cm. blood	% increase		Before	After	Before	After	Before	After
(a) Change from Air to N ₂													
16 {	C	3.5	37 min.	6	Nil Nil	-	Nil Nil	81.3	69.7	10.82	9.27	20.92	20.09
			1 hr. 1 min.	7		84.0		56.3	11.04	7.67	8.43	7.50	
17 {	C	2.8	8 min.	6	Nil Nil	-	Nil Nil	94.5	33.5	14.54	5.30	5.79	5.33
			1 hr. 35 min.	5		81.5		60.0	14.78	9.85	13.13	11.57	
25 {	C	3.8	36 min.	25	17.8-26.25 26.6-33.0	47.0	Nil Nil	95.5	73.0	14.76	11.38	16.93	16.79
			2 hr.	15		24.0		85.0	13.55	13.06	18.20	18.23	
15 {	N	2.8	2 min.	6	16.0-22.0 12.0-17.5	-	Nil Nil	-	-	-	-	-	-
			1 hr. 2 min.	6		-		-	-	-	-	-	
			2 hr. 23 min.	6		37.5		-	-	-	-	-	
23 {	C	3.1	42 min.	9		46.0		-	-	-	-	-	-
(b) Change from Air + 5% CO ₂ to N ₂ + 5% CO ₂													
23 {	C	3.1	27 min.	6	15.5-21.0 12.8-27.0 15.5-17.7	35.0	Nil Nil Nil	-	39.9	14.3	6.69	44.96	46.02
			1 hr. 34 min.	6.5		110.0		-	-	-	-	-	-
			2 hr. 19 min.	5		14.0		82.5	44.0	14.81	7.85	35.58	38.93
24 {	C	3.8	35 min.	3	20.6-29.0 16.8-20.0	41.0	Nil Nil	100.0	65.0	14.86	9.49	39.81	39.51
			41 min.	3		19.0		-	-	-	-	-	-

C = Chloralose.

N = Nembutal

4. The Effects of Nitrogen on Cat Lungs
(preliminary observations)

A pressor response to a change in the ventilating gas mixture to N_2 (from air) has been observed in 4 tests made in 3 experiments out of a total of 9 tests in 7 experiments (see Table VIa). All the tests were performed under negative pressure ventilation and the cat had been anaesthetised with chloralose before bleeding. The duration of the nitrogen inhalation varied from 6 to 25 minutes. Table VI shows that a considerable degree of desaturation of the pulmonary arterial blood occurred in every test in which blood samples were taken, and direct observation of the colour of the blood at the time of the experiment confirmed that the haemoglobin had become reduced in the other tests. The inconsistency of the results cannot therefore be attributed to a failure of the nitrogen to reach the alveolus. When the pressor response was present it appeared after a variable latent period (30 seconds to 10 minutes) and the pressure continued to rise as long as nitrogen was inhaled (see Fig. 15). On re-ventilation with air the pressure was restored to its original level within 5 to 10 minutes.

Tests have also been made of the effects of changing the gas mixture from 5 per cent. CO_2 in air to 5 per cent. CO_2 in N_2 . In these experiments an increase /

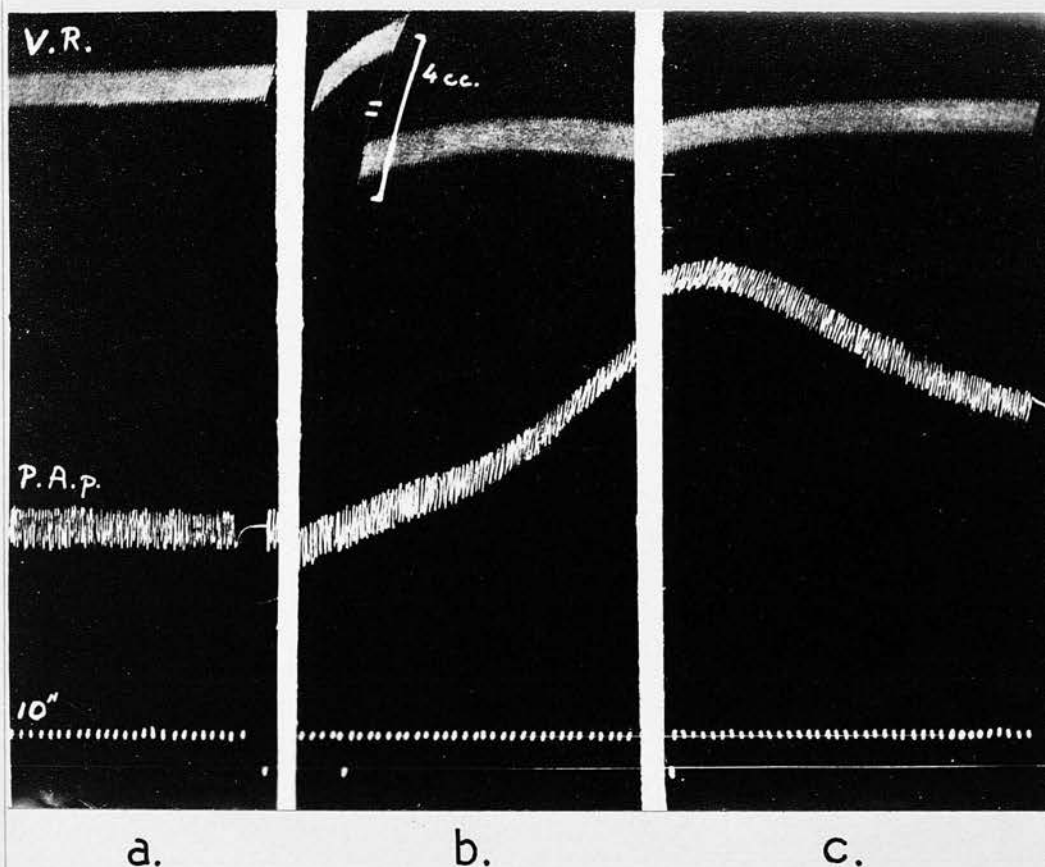


Fig. 16. C.I.P.L. 23. Cat φ 3.1 kg. Chloralose anaesthesia. Perfusion begun 11.20 a.m. Negative pressure ventilation 0 to -9 cm. H_2O .

a. 12.48 p.m. Ventilation on 5% CO_2 in air.

b. 12.54 p.m. Ventilation on 5% CO_2 in N_2 .

c. 1.00 p.m. Ventilation on 5% CO_2 in air.

Blood samples:

12.53 CO_2 35.58 vol.% O_2 14.84 vol.%

12.58 CO_2 38.93 vol.% O_2 7.85 vol.%

increase of pulmonary arterial pressure was consistently observed (see Table VIb). The pressure increased more rapidly than with pure N_2 , and the latent period for the response was shorter (20 to 40 seconds) see Fig. 16. The maximum pressure change which could have been produced by N_2 is probably much larger than these results would imply, since the pressure was always increasing rapidly when the lungs were reventilated with air.

No changes in lung blood volume were produced by nitrogen inhalation in the cat.

Pressor responses to the inhalation of nitrogen were not suppressed or reversed by dihydroergotamine in concentrations (1:50,000) which were sufficient to reverse the response to adrenaline.

V. SUMMARY OF EXPERIMENTAL FINDINGS

The effects of variations in the O_2 and CO_2 content of the gas mixture used for ventilation have been observed in isolated lungs of dogs and cats perfused through the pulmonary artery at constant volume inflow with the animal's own heparinised blood.

1. The Effects of CO_2 on Dog Lungs

- (1) If the concentration of CO_2 inhaled is increased from the small amount present in atmospheric air to 5 or 10 per cent., the capacity of the pulmonary blood vessels is markedly diminished and the pulmonary arterial pressure often shows a 5 to 20 per cent. increase.
- (2) The lung blood volume shows an inverse relationship to the CO_2 content of the arterial blood.
- (3) The effect of adrenaline on the pulmonary vessels is not altered during ventilation of the lungs with 5 per cent. CO_2 in air.
- (4) The response to CO_2 is not inhibited by ergotoxine or by atropine.

2. The Effects of O_2 , N_2 and CO_2 on Cat Lungs

- (1) Increasing the CO_2 concentration of the ventilating gas mixture from 5 or 10 per cent. produces a marked and consistent increase of pulmonary arterial pressure and the volume of blood in the lungs is sometimes diminished. This response is not inhibited by ergotoxine or atropine.
- (2) No effect is produced on the pulmonary arterial pressure or the lung blood volume by changing the ventilating gas mixture from air to O_2 . If the gas mixture is changed from 10 per cent. CO_2 in air to 10 per cent. CO_2 in O_2 there is sometimes a slight fall of pressure which might be accounted for by a decrease in blood CO_2 content.
- (3) If N_2 is substituted for air in the ventilating gas mixture the pulmonary arterial pressure is sometimes markedly increased. The exact conditions under which this pressor response occurs have not yet been elucidated. If the CO_2 concentration is kept constant at 5 per cent. and the gas mixture changed from 5 per cent. CO_2 in air to 5 per cent. CO_2 in N_2 a pressor response is /

is consistently seen. The pressor response to N_2 is not abolished by ergotoxine.

Nitrogen inhalation did not produce changes in the volume of blood in the lungs.

VI. DISCUSSION

In the conditions of these experiments, increasing the carbon dioxide concentration of the gas mixtures used for ventilation produces pulmonary vasoconstriction in isolated perfused lungs of dogs and cats.

In the dog lung inhalation of 5 or 10 per cent. CO₂ under the conditions defined, causes a marked diminution of the lung blood volume. The decrease in the capacity of the lung blood vessels in any one experiment is inversely related to the increase in CO₂ content of the pulmonary arterial blood (see Fig. 9 and Table III). The presence or absence of a slight increase in pulmonary arterial pressure in response to CO₂ in these preparations appears to be associated with the volume of the tidal air at the time of test and to the concentration of CO₂ inhaled (see p. 33).

In the lungs of cats, similar changes in CO₂ concentration consistently produce marked changes in pulmonary arterial pressure, and have little or no effect on the capacity of the pulmonary vessels.

Insufficient evidence is available at present to ascertain whether this is a true species difference or whether it arises from a difference in the lung blood volume relationships in the apparatuses. If there is
a /

a true species difference it may mean that different blood vessels react in the two species or, if the same vessels respond, then they must have different pressure-volume relationships. Gaddum and Holtz (1933) reported variations in the response to drugs of the pulmonary blood vessels of dogs and cats and Daly (1938) found that the responses of the lung blood vessels of dogs, guinea pigs and Macacus rhesus differed from each other in many respects.

In both cat and dog lungs the effects produced by CO₂ are not reversed or suppressed by ergotoxine and are not affected by atropine. This indicates that adrenergic and cholinergic nervous elements are not involved and the effect is probably exerted directly on the vessel wall.

Previous investigators have reported pressor responses to CO₂ in the isolated perfused lungs of dog (Binet and Bourlière, 1941) cat (Nisell, 1948) and Macacus rhesus (Hebb and Nimmo Smith, 1949). Binet and Bourlière have however reported that CO₂ produces an increase of lung blood volume if it is inhaled in concentrations of less than 50 per cent. The reason for the discrepancy between their results and those now reported is obscure. Anaesthetisation of the animal with chloralose is apparently not a contributing factor. Binet and Bourlière found much greater /

greater increases of pulmonary arterial pressure than those which were obtained in these experiments, a fact which may be correlated with their larger tidal air volume (see Binet and Bargeton, 1939). It was thought that the air cushion on the output side of the pump in their apparatus might lead to errors in the measurement of the volume of blood in the venous reservoir because a sudden increase of pulmonary arterial pressure would tend to increase the volume of blood in the air cushion. However, on testing this possibility by using a similar air cushion with the Dale-Schuster pump no such capacity effect could be obtained by me. Ketcham, King and Hooker (1912), who perfused the lungs of rats at constant pressure, attributed the increase in venous outflow produced by CO_2 to a vasodilatation in the lungs. A progressive vasoconstriction might, however, have this effect under certain conditions.

In the tests made on cat lungs it was not found that a change in the ventilating gas mixture from air to pure O_2 produced any characteristic pulmonary vasomotor response. The slight inconstant decrease in pulmonary arterial pressure following a change from 10 per cent. CO_2 in air to 10 per cent. CO_2 in O_2 was not more than could be accounted for by the slight decrease in blood CO_2 content which also occurred.

The /

The response to oxygen lack was also inconstant, although further work clearly needs to be done on this point. It appears likely that vasoconstriction occurs in some part of the pulmonary vascular bed when the oxygen tension of the blood is reduced, but it is not possible to decide whether this response is solely due to anoxaemia or to some other factor. It remains to be discovered if the inconstancy of the response to oxygen lack in isolated perfused lungs in comparison with the response obtained in the intact animal (Von Euler and Liljestrand, 1946b) is due only to the conditions of the isolated organ or if the response in the intact animal is conditioned by mechanical or other factors. According to Nisell (1948) a pulmonary pressor response to N_2 in isolated lungs is only seen when ventilation is by negative pressure. No experiments have yet been done to confirm this statement. These experiments give no support to the contention (Von Euler and Liljestrand, 1946b; Logaras, 1947) that oxygen has a vasodilating effect on the lungs blood vessels. This discrepancy might be due to the fact that the pulmonary arterial blood in the isolated organ has an abnormally high O_2 content, but slight anoxaemia occurring in the intact animal might also be responsible. Nisell (1948) apparently could only produce a fall of pulmonary /

pulmonary arterial pressure as a result of O_2 inhalation if the gas mixture had previously been N_2 .

It is believed that isolated lungs are suitable preparations on which to test the effects of respired gases on the pulmonary vascular bed, for not only can the conditions of ventilation and circulation be absolutely controlled but it is also possible to test the responses without the intervention of nervous and humoral mechanisms. In no previous investigation have the changes in blood gas concentration been determined simultaneously with the pulmonary vasomotor effects. This important control is relatively easy to perform under the conditions of the isolated organ.

However, even in this preparation certain disadvantages are inherently present. One of the most important of these is the abnormal state of the blood gas concentration. It has been shown (see p. 34) that if isolated lungs are ventilated with air then the CO_2 content of the pulmonary arterial blood falls rapidly and may reach as low a level as 3.0 vol. per cent., while the arterial blood O_2 concentration remains high. The pulmonary vessels in the intact animal are thus in very different conditions, and further evidence requires to be obtained before the responses of the isolated lung can be accepted as a normal occurrence. It might be possible to perfuse the /

the left lung separately in a whole animal after the method (Daly and Duke, 1948) described for the dog. This method would ensure that the blood arriving at the pulmonary vessels would be normally reduced. Alternatively, a double lung perfusion could be set up, in which the first lung would respire gas of low O_2 and high CO_2 content and the venous outflow from this lung be passed into the pulmonary artery of the second lung which would be ventilated with air or a test gas mixture. This method would ensure that the gaseous exchange of the second lung would be nearly normal.

It is of interest that, if the pulmonary vessels constrict to CO_2 and N_2 , then in this respect they differ markedly from the vessels of the systemic circulation. A large amount of evidence is available showing that peripheral systemic vasodilatation occurs in denervated vessels after the inhalation of gas mixtures containing 5 to 20 per cent. CO_2 (Mathison, 1910; Fleisch 1918, 1921; Dale and Evans, 1922). Krogh (1922) and Atzler and Lehmann (1921a and b, 1922) showed that a similar vasodilatation occurred in warm and cold-blooded animals as a result of increasing the hydrogen ion concentration of the perfusate. Anoxaemia has also been thought to have a peripheral systemic vasodilating effect (Mathison, 1910; Krogh, 1922). It may be that the discrepancy between /

between the response of the pulmonary and systemic circulations lies not in a true difference in reactivity but in the conditions of the experiments. For instance, gross changes in blood pH may produce vasoconstriction instead of vasodilatation (Atzler and Lehmann, 1921b, 1922) but the position is not all clear for a systemic vasodilatation occurs most readily when the perfusate has a low buffering power. It is apparent that changes in blood gas concentration may be misleading since they do not necessarily indicate the true state of the interior of the cell. Bernthal (1930) recorded an initial vasoconstriction to the inhalation of 10 per cent. CO_2 in the denervated brachial and femoral arteries of the anaesthetised cat, this response gave place to a vasodilatation after 2 hours. The superior mesenteric artery always showed a dilator response.

Before discussing the possible physiological significance of the effects produced by CO_2 and O_2 on the pulmonary blood vessels it is important to decide which of the vessels take part in the response. Very little information is available on this point. In isolated dog lungs the absence of a consistent pressor response to CO_2 suggests that the arterioles are not primarily involved. The short latent period before the beginning of the response which was only half /



half to one-sixth of the time required for a complete circulation of the blood in the apparatus also appears to exclude an initial action of CO_2 on the arterioles. It is not considered likely that the large changes in lung blood volume could be accounted for by a capacity effect due to venous constriction alone, and the simplest explanation of the action of CO_2 is that it causes constriction of the capillaries with or without a concomitant contraction of the veins. More complex effects involving a simultaneous opening and closing of different parts of the pulmonary vascular bed with a redistribution of blood within the lungs can also be visualised. In the cat isolated lung the constancy of the pressor response to CO_2 may mean that the arterioles constrict, but the circulation time of the blood in the apparatus was too rapid to allow any definite conclusion to be drawn from the length of the latent period of the response. Capillary constriction also seems to be the explanation for the pressor effect produced by O_2 lack and CO_2 excess in Von Euler and Liljestrand's experiments (1948). For in these the latent period of the response was often under 30 seconds which is a shorter period than the circulation time in the cat (Doi, 1921). Dirken and Heemstra's results (1949) could also be explained on the basis of capillary constriction. They observed that if one lung /

lung respired a gas mixture of low O_2 content and the other a gas mixture of high O_2 content then the circulation was gradually adjusted in favour of the lung respiring and a high O_2 content. Further, the lung respiring low O_2 mixtures became pale and its oxygen uptake was reduced. However, the extremely long period (4 to 8 hours) for the process to become established prompts the suggestion that some factor such as the production of toxic substances in the anoxic lung may be concerned.

The Possible Physiological Significance of These Reactions

Hochrein and Keller (1932) have produced evidence that the lungs may be an important blood reservoir, with functions very similar to those envisaged for the spleen by Barcroft (1934). The present observations confirm their findings and also those of Sjöstrand (1935) that the lung blood volume is diminished by the inhalation of excess CO_2 . This would imply that during hypercapnia and possibly also during anoxia, the circulating blood volume would be increased, with increase of cardiac output and increased opportunities for gas exchange. The suggestion (Von Euler and Liljestrand, 1946b) that the pulmonary vasoconstriction that occurs, both as a response to oxygen lack and excess CO_2 inhalation is part of a mechanism for diverting /

diverting blood from unventilated parts of the lung, can only be accepted if it can be proved that the capillaries and venules take part in the response.

Since in these conditions all blood in the pulmonary arteries would have the same blood gas content.

Dirken and Heemstra (1949) have shown that during prolonged anoxia in the rabbit the blood is apparently diverted from a lung breathing nitrogen.

In view of the findings that changes do occur in pulmonary vasomotor activity if the normal constituents of the alveolar air are altered in concentration, the possibility of a reflex regulation of respiration by peripheral receptors in the lung must be considered. Pi Suner and Raventos (1931 - 1933) performed cross circulation experiments in which the blood of the dog B was perfused with the blood of another dog A. When the trunk of B was given gas mixtures containing excess CO_2 the respiratory movements in the head were stimulated. This stimulation occurred after the trunk of B had been bled out and the lungs only perfused with a Dale-Schuster pump. These findings apparently contradict the allegation (Heymanns and Heymanns, 1927) that the peripheral respiratory reflexes arise only from the carotid and aortic receptors. Dirken and Dishoeck (1937) also found evidence of a peripheral stimulation of respiration by CO_2 which was dependent on the integrity of /

of the vagi. The existence of chemoreceptors in the lungs has not been demonstrated on experimental or histological grounds, and there is no evidence which would implicate the Hering-Breuer receptors in these responses. Indeed, the work of Adrian (1933) and Partridge (1933) is seriously against this latter possibility, for they found that CO₂ inhalation in the cat and the rabbit did not alter the frequency of the impulses from the stretch receptors, recorded in the cervical vagus. The possibility remains, therefore, that the peripheral reflex stimulation of respiration observed by Pi Suner has its origin in pressor receptors in the pulmonary vascular bed. Physiological evidence for the existence of such receptors has been obtained by Daly et al. (1937), Whitteridge (1948) and others. Further work must be done before the importance of such mechanisms can be assessed.

It is of interest to consider whether the pulmonary vascular reactions to variations in alveolar CO₂ and O₂ could have any influence on gas exchange in the lungs. But speculation on this problem is fruitless in the absence of more precise knowledge of the time relationships of the process of the oxygenation of the haemoglobin in the alveolar capillaries.

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PART II.

CHANGES IN RENAL FUNCTION

DUE TO

THE ACTION OF CERTAIN DRUGS

UPON THE HYPOTHALAMUS.

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I. INTRODUCTION

One of the functions of the mammalian kidney is to assist in maintaining the water balance of the body by varying both the rate of urine flow and the tonicity of the urine.

It is generally accepted that the initial process of urine secretion is a physical filtration occurring across the glomerular membrane. The plasma ultrafiltrate so formed is elaborated into urine during passage through the tubules by the reabsorption of some substances into the peritubular blood and the excretion of others from the blood into the tubules. The filtration reabsorption hypothesis was first advanced by Ludwig (1844) and supported by Cushny (1917). It has been directly proved in the frog and necturus (Richards, 1929) and indirect evidence obtained in mammalian kidneys is also in favour of such a process (Winton, 1937).

In this thesis it is proposed to discuss changes in the rate of urine flow produced as a result of variations in the amount of antidiuretic hormone released from the pituitary gland. It is important, first, to discuss other possible factors which may influence the rate of urine flow.

(1) The glomerular filtration rate.- There is no evidence in man and dog, that the rate of glomerular filtration, /

filtration, obtained from the inulin clearance, bears any constant relationship, in normal conditions, to the rate of urine flow (Chasis, 1937). This may not be true of other species, however, for in the rat and the rabbit there is apparently a direct relationship between the glomerular filtration rate and the rate of urine secretion (Kaplan Smith, 1935). This latter relationship also holds good in the isolated kidney (Winton, 1937).

(2) The osmotic pressure of the fluid in the tubules.- The reabsorption of water in the tubules must be an active process since it works against the osmotic gradient between the blood and the tubular fluid. The introduction into the blood and the glomerular filtrate of any osmotically active substance which is not reabsorbed in the tubules, or is only slightly reabsorbed will tend to increase the rate of urine formation by passively preventing the reabsorption of water.

(3) The Renal Nerves.- There is no evidence that denervation of the kidneys affects in any way the power of the kidney to excrete water, and a denervated kidney responds by diuresis to the ingestion of water in a similar way to an innervated kidney (Klisieki, et al., 1933; Rustrum, 1943).

(4) /

(4) Drugs and Hormones. - Any substance which influences the metabolism of the tubular cells may also affect the rate of water excretion. The administration of many metallic salts is, for instance, followed by a diuresis. Evidence will be presented later that the antidiuretic hormone has a direct effect on the tubules leading to an increased reabsorption of water. It is important to emphasise that the absolute amount of water which is available for reabsorption in this way is but a small fraction of the volume of the glomerular filtrate. The glomerular filtration rate remains remarkably constant at about 120 c.c./1.73 sq.m./min. in man and yet the fastest rate of urine secretion is probably not more than 20 c.c. per minute, and the resting rate is much lower. This implies an obligatory reabsorption of at least 100 c.c. per minute, and it is an example of the conservative powers of the kidney.

The Part Played by the Pituitary
and Hypothalamus in Water

Excretion

Schäfer and Magnus (1901) and Schäfer and Hering (1906) were led from their experiments on anaesthetised animals to believe that extracts of the posterior lobe of the pituitary gland had a diuretic action. This view was shared by Frank (1912). V. den Velden (1913) /

(1913) and Farini (1913) first showed that posterior pituitary extract was effective in controlling the symptoms of diabetes insipidus in man. Since that time much evidence has accumulated showing the true antidiuretic nature of the extract.

It is now generally accepted that the neurohypophysis secretes an antidiuretic hormone which acts directly on the kidneys promoting the absorption of water in the tubules. The evidence for such a view has been discussed at length by Fisher, Ingram and Ransom (1938), Pickford (1945), O'Connor (1947), Verney (1947) and Harris (1948). It is, therefore, only proposed to summarise here some of the most important points relating to the nature of the antidiuretic hormone and to the control of its secretion.

Although the precise structures which manufacture the hormone are unknown there is evidence that it is formed in the neurohypophysis and not the adenohypophysis. This is derived from the results of biological assays of the antidiuretic properties of extracts of the various lobes of the hypophysis.

Extracts of the neural lobe have greater antidiuretic properties than do extracts of the pars intermedia (Van Dyke, 1926) and extracts of the glands of birds and whales in which species the pars intermedia is lacking, or separate from the neural lobe, also have antidiuretic /

antidiuretic properties (Geiling and Robins, 1918). After the atrophy of the renal lobe which follows secretion of the pituitary stalk, (an operation which leaves the pars intermedia normal) extracts of the posterior pituitary gland have no antidiuretic activity (Fisher, et al., 1938).

In this thesis the terminology used for the various subdivisions of the pituitary gland will be that used by Rioch, Wislocki and O'Leary (1940).

The hormonal nature of posterior pituitary extracts is demonstrated by the fact that their effects are exerted independently of the integrity of the renal nerves, and they inhibit the excretion of intravenously administered water, while they do not inhibit the absorption of water from the intestine (Klisiaki, et al. 1933). The effects can also be exerted independently of changes in renal blood flow or systemic blood pressure (Cowan, Verney and Vogt, 1938). The extract is also effective in relieving the symptoms in conditions arising from a deficiency of the hormone. Further evidence, to be discussed later, indicates that the hormone can be released from the gland in response to normal stimuli.

The chemical nature of the substance is unknown, and so far attempts to extract it separately from the pressor fraction have not been completely successful (Stehle and Fraser, 1935).

A deficiency of antidiuretic hormone is characterised by the secretion of large volumes of urine of low specific gravity and low chloride content. These symptoms are present in diabetes insipidus, and in the isolated kidney. In both conditions the injection of posterior pituitary extract lowers the rate of urine excretion and increases the rate of chloride excretion. A urine of similar characteristics is also secreted during water diuresis in man and other animals, and in this condition also, the rate of urine flow is diminished and the rate of chloride excretion increased by the injection of extracts of the posterior pituitary gland. The effect of posterior pituitary extracts in water diuresis prompted Verney's suggestion that the state of water diuresis was due to a diminished output of the antidiuretic hormone from the pituitary gland. Verney and his co-workers (Klisieki, et al., 1933) found that after the ingestion of water there was a latent period of approximately 15 minutes between the time taken for a maximum water load to be absorbed and the time taken for the urine to reach a maximum rate of excretion. They interpreted this finding as indicating the time taken for the circulating hormone already present in the blood or tissues to be used up.

The type of inhibition of urine flow produced by the injection of posterior pituitary extracts during water diuresis shows certain characteristics. The maximum /

maximum diminution of the rate of urine excretion is attained in 3 to 10 minutes and thereafter there is a slow recovery of the rate of urine flow. The control rate is obtained within 30 to 40 minutes. This type of inhibition will be referred to in this thesis as the "pituitary type". It is very different from the change in rate of urine flow obtained by compression of the renal artery (Verney, 1946), or by the injection of adrenaline. In the latter condition the rate of urine flow is sharply diminished and the control rate is attained within 8 minutes, in the former condition the inhibition of urine flow lasts only for as long as the compression is continued.

The Hypothalamic Connections of the Neurohypophysis

The existence of nerve tracts between the neurohypophysis and certain hypothalamic nuclei has been confirmed by histological examination of normal stained specimens, and also of specimens in which previous injury had been inflicted in the course of the tracts.

Cajal (1894) first found evidence of an unmyelinated tract running in the infundibular stalk from the supraoptic nucleus to the neural lobe in young mice. The existence of this tract has been confirmed in many other species (see review by Fisher, et al., 1938). Section of the infundibular stalk or removal of the neural lobe causes retrograde degeneration in the cells /

cells of the supraoptic nucleus. O'Connor (1946) found that, in dogs, approximately 75 per cent. of the supraoptic nuclear cells degenerated after removal of the neural lobe, and section of the infundibular stalk at the level of the median eminence resulted in a loss of 80 to 95 per cent. of the cells. Similar figures have been obtained in the monkey (Fisher, et al., 1938). If the supraoptico-hypophysial tract is sectioned rostral to the median eminence degeneration occurs not only in the neural lobe but also in the infundibular stalk and the median eminence, this indicates that all these structures are innervated from the supraoptic nucleus.

Greving (1923) showed that the paraventricular nuclei were linked to the supraoptic nucleus and neural lobe, and it has since been found (Heinbecker and White, 1941) that nearly complete removal of the neurohypophysis results in retrograde degeneration of this nucleus.

Fisher, Ingram and Ranson (1938) also describe another tract, the tubero-hypophysial tract which appears to arise from the lateral and caudal perinfundibular regions.

Other possible nervous connections of the neurohypophysis have also been described, and connections of the hypothalamus with the thalamus and cerebral cortex have been suggested, but little is known about these /

these (see review by Pickford, 1945).

Sympathetic fibres enter the neural lobe with the posterior median hypophysial artery (Dandy, 1913) and a parasympathetic supply to the posterior lobe has also been described. Further important information as to the nerve supply of the neurohypophysis is derived from the results produced by lesions inflicted at some point on the tracts between the hypothalamus and the neural lobe.

The Production of Diabetes Insipidus

Although isolated instances of removal of the neural lobe in dog and man (Fisher et al., 1938) are recorded as being followed by diabetes insipidus, there is little adequate histological evidence to ensure that hypothalamic injury did not also occur. Most observers are now agreed that a state of permanent polyuria does not follow this operation in the cat (Fisher et al., 1938) or dog (O'Connor and Verney, 1945; Pickford and Ritchie, 1945; Heinbecker and White, 1941). Diabetes insipidus can, however, be produced in rats after removal of the neural lobe (Richter, 1934). The observation that a hypothalamic injury could lead to a permanently increased fluid exchange was first made by Camus and Roussy (1913), and the position has been considerably clarified by the work of Fisher et al. (1938). These latter workers inflicted carefully controlled lesions in various places /

places in the hypothalamus of the monkey and cat. They found that section of the supraoptico-hypophysial tracts was followed by a permanent polyuria if the interruption was placed rostral to or in the median eminence and approximately half the fibres were cut on both sides. They were led to regard the neurohypophysis as consisting of the neural lobe, the median eminence and the infundibular stalk, all three of which portions are capable of secreting the anti-diuretic hormone, and all three of which had to be removed or denervated before a deficiency state could be produced. Their findings have been confirmed in the dog by White and Heinbecker (1941), Pickford and Ritchie (1945) and O'Connor (1946). In dogs the survival of approximately 15 per cent. of the normal number of supraoptic nuclear cells is sufficient to prevent the appearance of diabetes insipidus (Heinbecker and White, 1941).

Complete hypophysectomy does not usually produce diabetes insipidus in the dog, cat or monkey (Fisher et al, 1938; Heinbecker and White, 1941; Pickford and Ritchie, 1945). The reason for this remains obscure at present. A diuretic effect of anterior lobe extracts was observed by Crowe, Cushing and Homans (1909), and it has been suggested that complete hypophysectomy, producing as it does a loss of the anterior lobe as well as the posterior lobe will interfere with /

with the power of the kidney to exhibit a maximum diuresis. Complete hypophysectomy, however, produces a profound depression of metabolism with a decrease in cardiac output and a decrease in renal blood flow. It is uncertain how far these factors are responsible for the prevention of the appearance of diabetes insipidus after removal of the whole gland.

Comment

For the sake of clarity and brevity, no discussion has been made of certain findings, which may be very important, but they are difficult to fit into the picture as it appears at present. The hypothesis of a "water centre" in the medulla which was advanced by Molitor and Pick appears to have been satisfactorily disposed of by Theobald (1934). Certain workers are in favour of a secretion of an antidiuretic hormone outside the neurohypophysis (Melville and Hare, 1945; Keller, 1942). The findings of Newton and Smirk (1934) and Fee (1929) indicate that diuresis in response to ingested water can occur in a relatively normal manner in decerebrate and hypophysectomised animals, and that the presence of an intact neurohypophysis may not be essential for this process. This work requires further confirmation and study.

The Control of the Output
of the Antidiuretic Hormone

If stimuli which produce changes in the rate of urine flow are to be regarded as effective in causing variations in the rate of release of the antidiuretic hormone from the neurohypophysis, then some or all of the following conditions must be fulfilled:-

- (i) The stimulus should be ineffective, or its efficacy should be much reduced after removal of the neural lobe of the hypophysis.
- (ii) Denervation of the kidneys or suprarenals should not reduce the efficacy of the stimulus, and the effects of the stimulus should not be dependent on changes in blood pressure or renal blood flow.
- (iii) It should be possible to match the response obtained by a stimulus with that obtained by a suitable dose of posterior pituitary extract.

Using the above criteria, it has been possible to demonstrate that the output of the antidiuretic hormone can be varied in a number of conditions: these have been reviewed in detail (Pickford, 1945) and mention will only be made of those findings which either appear outstandingly important or which have a direct bearing on the subject of this investigation.

1. Variations in the osmotic pressure of the blood.- Verney (1947) has made a comprehensive study of the effects of acute changes in blood electrolyte content on the rate of urine flow during water diuresis. In the normal dog intracarotid injections of hypertonic solutions produced an inhibition of urine flow rate of the "pituitary type", this effect was much reduced after removal of the posterior lobe of the pituitary gland. Isotonic solutions of NaCl, Na₂SO₄ and sucrose had similar effects, but urea was less active and dextrose least active of all. Verney postulates that the effect is exerted on specialised receptors, "osmoreceptors", which have a selective permeability to certain salts. The precise site of the osmoreceptors is unknown, but they lie somewhere in the territory of the internal carotid artery, and Verney suggested that they were possibly contained in the supraoptic nucleus. That this mechanism may be operative under normal conditions is shown by the fact that changes in osmotic pressure of the blood of the order of 1 per cent. can cause measurable changes in the rate of water excretion.

There is no indication, from Verney's experiments, that accommodation of the "osmoreceptors" occurs during 40 minute exposures to an increase in their osmotic environment, but Baldes and Smirk (1934) found evidence from more chronic experiments that some degree of accommodation could occur.

As /

As further evidence that variations in the osmotic pressure of the blood are effective in causing an increased output of antidiuretic hormone is the observation (Gilman and Goodman, 1937) that the urine of dehydrated rats contains an antidiuretic substance which is not present in the urine of normal rats or in the urine of dehydrated hypophysectomised rats.

Chambers (1945) describes changes in the cellular structure of the neural lobe following administration of salt to rats.

2. Direct stimulation.- Recent observations (Harris, 1947) show that direct stimulation of the hypothalamic region in rabbits can produce an inhibition of urine flow of the pituitary type, which can be matched with an injection of posterior pituitary extracts.

3. Exercise and emotion.- A full account of the early work describing the diminution of urine flow that occurs during emotional stress is contained in a paper by Verney (1947). Verney and his co-workers (Rydin and Verney, 1938; Cowan, et al. 1938; O'Connor and Verney, 1942) have shown that the slow "pituitary type" of antidiuresis which may be produced as a result of exercise or emotion, is not dependent on the integrity of the renal or splanchnic nerves or changes in renal blood flow. They observed that in some dogs an emotional stimulus only produced a brief /

brief diminution of the rate of urine flow. In these animals section of the splanchnic nerves and denervation of the kidneys and suprarenal glands was followed by the production of a typical slow "pituitary type" of inhibition in response to emotional stress. This finding induced them to attempt to inhibit the appearance of the slow inhibition by the injection of adrenaline. It was found that small doses of adrenaline (5 to 30 μ g. intravenously) were effective in preventing the emotional antidiuresis, if the adrenaline was given 30 seconds before the application of the stimulus.

4. Drugs.- Pickford (1939) found that injections of acetyl choline into the normal, unanaesthetised, atropinised dog during water diuresis produced an inhibition of urine flow of the pituitary type. This effect was unchanged after denervation of the kidneys and it was much reduced after removal of the lobe of the pituitary gland. The inhibition of urine flow was not dependent on changes in blood pressure and, while it could be matched with suitable doses of pituitary extract, it could not be simulated either by adrenaline or by amyl nitrite. The inference was that acetyl choline acted on some part of the chain of neurones connected in the release of the antidiuretic hormone. More recently, Pickford (1947) has shown that acetyl choline injected into the supraoptic nucleus /

nucleus of the chloralosed dog also produces a similar inhibition of urine flow. This effect is potentiated by eserine. Control injections of adrenaline or saline in the same region are without effect.

The effect of intravenous injections of acetylcholine in suppressing the rate of urine flow during water diuresis suggested that nicotine might have a similar action. This has been found to be the case in rats and man (Burn, Truelove and Burn, 1945).

Observations have also been made that indicate that morphine may stimulate the output of the anti-diuretic hormone. De Bodo (1944) found that intramuscular or intravenous injections of morphine (dose - 2.5 to 5 mg./kg.) to dogs decreased the volume of urine excreted in three hours after a test dose of water. This effect was not present after removal of the posterior lobe of the pituitary gland.

Fugo (1944) describes an antidiuretic effect of yohimbine in normal dogs, and Ssargin and Nussimboim (1937) find that atropine diminishes the rate of water excretion in mice. This latter finding is not confirmed in the dog (Pickford, 1939).

Summary /

Summary

The neurohypophysis secretes an antidiuretic hormone which has a direct action on the kidney promoting the reabsorption of water.

Nervous connections exist between the hypothalamus and the neurohypophysis. The most important of these appears to be the supraoptico-hypophysial tract, which connects all three parts of the neurohypophysis (the median eminence, the infundibular stem and the neural lobe) with the supraoptic nucleus. Section of the supraoptico-hypophysial tract results in degeneration of the neurohypophysis and the supraoptic nucleus, and diabetes insipidus can be produced in this way, provided the lesion is bilateral and over half the fibres in the tract are sectioned.

Stimulation of the supraoptic nucleus can increase the output of the antidiuretic hormone, and it must be assumed that the neurohypophysis is innervated from the supraoptic nucleus.

The significance of the other nervous connections which have been described is not so clear.

The output of the antidiuretic hormone can be stimulated by various methods. The antidiuresis of emotional stress and that due to an increase in the osmotic pressure of the blood may occur under normal conditions.

It is suggested that acetyl choline acts as the transmitter in at least one part of the chain of neurones which control the output of the hormone.

II. METHODS

The experiments have been of two types. (a) In chronic experiments observations on the same animal have been made at intervals for periods extending over several months. The observations have all been made during water diuresis, and tests have been made of the effects produced by various drugs on the rate of urine flow. (b) In acute experiments, performed on animals under chloralose anaesthesia and during water diuresis: the effects of drugs injected into the supraoptic nucleus or intravenously have been observed.

(a) Chronic Experiments

Six healthy bitches (weight, 7.0 to 20.0 kg.) have been used. A description of the animals and their histories are given in the appendix. In addition, one animal (M.59, Buster) was observed for a short period during recovery from an acute operation which had involved the injection of acetyl choline into the supraoptic nucleus. Four animals (Spot, Poppet, Snapper and Trix) were observed for several months after the injection of D.F.P. (diisopropyl fluorophosphate) into the supraoptic nucleus. In three normal animals (Wolf, Buster, Brownie) and also in Poppet and Trix a preliminary operation was performed under nembutal anaesthesia, and with full aseptic /

aseptic precautions, one to two weeks before any observations were made. At this operation the kidneys were denervated by a thorough stripping of their pedicles. With the exception of Wolf and Poppet, all the dogs had a preliminary perineotomy operation, also performed under nembutal anaesthesia, in which the dorsal wall of the vagina was slit to expose the urethral opening and facilitate catheterisation. In Blackie, the right suprarenal gland was removed and the left gland denervated at a two-stage operation.

As far as possible the time table of an experiment was as follows:- On the morning of an experiment the dog was given a hydrating dose of 200 c.c. of water by oesophageal tube and the water bowl was removed from the cage. Two and a half to three hours later, when the dog was in a Pavlov stand, the bladder was catheterised and drained with a self-retaining rubber catheter (size 4 to 6). 250 to 300 c.c. of warm water were then given by oesophageal tube according to the size of dog. The volume of urine subsequently passed was recorded every 15 minutes for the first 30 to 45 minutes and then every 3 to 5 minutes. The rate of urine flow was recorded in c.c. per minute.

This method of recording the urine flow rate was found to be sufficiently accurate provided the bladder was drained before the volume of each sample was measured. /

measured. At the peak of the water diuresis the variation in the urine flow rate over successive three minute periods was less than 0.3 c.c. per minute. Greater constancy than this would not be expected since the urine flow from the kidney itself is intermittent (Klisiaki, et al. 1933).

Atropine sulphate (0.75 to 2.0 mg. according to the weight of the animal) was injected subcutaneously within the first 20 minutes of the experimental period in all experiments in which acetyl choline was given. Atropine was not given to the same dog on more than three successive days because of the danger of atropine intoxication. No symptoms of this were observed during the present investigation. When eserine was used 1.0 to 2.0 mg. eserine salicylate were injected subcutaneously 20 minutes from the beginning of the experiment, or at least 20 minutes before a test dose of acetyl choline.

Intravenous injections of drugs.- All drugs for intravenous injection were diluted with 0.9 per cent. saline so that the final volume to be injected was 0.5 c.c. The injections were made into the saphenous vein using fine hypodermic needles (size 19 or 20). The injections caused no objective disturbance to the dogs and control injections of saline had no effect on the rate of urine flow (see Fig. 6).

(b) /

(b) Acute Experiments

Healthy bitches (weight 5 to 17 kg.) were used in which the kidneys had been denervated 7 to 10 days previously. The day before the experiment the pituitary was exposed by the transphenoidal route (Pickford, 1939) and a perineotomy was performed.

On the morning of the experiment the dog was usually in very good condition. It was given a hydrating dose of 1 pint fluid (half milk, half water) at 9.0 a.m. Two to two and a half hours later chloralose (0.14 to 0.19 g./kg. body weight) was given in 250 to 350 c.c. water by oesophageal tube. The bladder was then catheterised and urine collection begun.

As soon as anaesthesia was sufficiently deep the animal was placed on its side on an inclined board and the mouth held open with clamps (see Pickford, 1947).

Injections into the supraoptic nucleus.- With the soft palate retracted it was possible to see the previously exposed pituitary and optic chiasma. The injections were made into the supraoptic nucleus with a long, fine, short bevelled needle, bent at an obtuse angle 0.5 cm. from its tip. A 1 c.c. "Record" syringe was used to the end of which was attached a screwhead such that each quarter turn delivered 0.002 c.c. from the needle.

Recording /

Recording of blood pressure.- In two experiments the blood pressure was recorded from a cannula in the femoral artery and one of its main branches. A mercury manometer was used with saturated sodium sulphate solution as an anticoagulant.

Renal Clearances

Measurements of the diodone and creatinine clearances were made at intervals during all the acute experiments and in one or two other experiments.

9 to 18 c.c. of a 35 per cent. solution of diodone (Pyelosil, Glaxo) was injected subcutaneously half to one hour before the chloralose was given or at the same time interval before the second dose of water if no anaesthesia was used. At the same time 3 to 4 g. creatinine were given in 100 c.c. water by oesophageal tube. Blood samples from the saphenous vein were taken at intervals of not more than one hour during the experimental period. The samples were centrifuged and the concentration of diodone in the serum estimated by the method of Alpert (1941), the serum creatinine concentration was estimated by the alkaline picrate method. Urine samples were taken at various intervals (3 to 30 minutes) according to the rate of urine flow and the conditions of the experiment and the concentration of creatinine and diodone in the urine estimated. The serum concentration of creatinine /

creatinine and diodone at the midpoint of each urine sample collection period was then extrapolated from a graph. The clearance of each substance was determined using the formula devised by Van Slyke, et al. (1928):-

$$\text{Clearance} = \frac{UV}{P} \text{ where } U = \text{concentration of substance in urine.}$$

P = concentration of substance in serum.

V = rate of urine flow in c.c./min.

All the clearances were reduced to a surface area of 1.0 sq.m. by the use of Meeh's formula (Lusk and Dubois, 1924). Surface area in sq.m. - $K^3 \sqrt{W^2}$.
Where K = 11.2 and W is the weight in kg.

The clearance of creatinine has been shown to equal that of inulin in the dog (Van Slyke, et al. 1935) and it was chosen in preference to inulin in this series of experiments because of the convenience of administration. The creatinine clearance is thus equal to the glomerular filtration rate. Provided certain precautions are taken the plasma clearance of diodone is equal to the total effective plasma flow, although because diodone diffuses through the membrane of the red cell more readily in dog than in man, the diodrast clearance is perhaps less accurate a test in the dog than in man (White and Heinbecker, 1940). In these experiments the concentration of diodone in the /

the blood was always well below the level (13 mg. diiodone I_2 per cent.) at which depression of clearance is seen, and relatively constant plasma levels of the substances were maintained throughout the experimental period.

As a test of the accuracy of this method successive control clearances gave results which were substantially similar (with variations of about 20 c.c.).

Histological specimens.- At the end of an acute experiment the animal was killed by a stab wound in the chest before it had recovered from the anaesthetic. The vault of the skull was removed together with most of the cerebrum. The base of the skull with the hypothalamus and pituitary in situ was left to harden in 5 per cent. formal saline for 24 hours, before being dissected out. Serial sections (10 μ) were cut of the hypothalamus and pituitary gland and stained with toluidine blue. Every fifth section was examined, and when cell counts of the supraoptic and paraventricular nuclei were made only those cells containing nucleoli were counted. Three of the dogs on which chronic observations were made (Spot, Poppet and Brownie) were killed under nembutal anaesthesia. The head was perfused with normal saline and then with a solution of 5 per cent. formal saline containing 10 per cent. saturated $HgCl_2$ solution. The head was left for 24 hours and then treated as above.

III. RESULTS

1. The Effects of Intravenous Acetyl Choline on the Unanaesthetised Atropinised Dog

In confirmation of Pickford (1939) it has been found that intravenous injections of acetyl choline produce an inhibition of urine flow in the normal unanaesthetised dog during water diuresis.

Acetyl choline (Roche) from a fresh ampoule, was diluted with 0.9 per cent. saline 10 to 15 minutes before use so that the final volume injected was 0.5 c.c. In some cases the final dilution was made from a stock solution of 1.0 per cent. acetyl choline in 5.0 per cent. NaH_2PO_4 . The stock solution was kept in a refrigerator for periods of up to 14 days. No variation in response was noted which could be attributed to this difference. The injections were made into the saphenous vein on either side.

The response to acetyl choline has not been so constant as that originally described (see p. 15) either with regard to the number of times acetyl choline was effective or to the size of dose necessary to produce a response. Of the 7 animals used in this investigation (Blackie, Brownie, Wolf, Buster, Cleo, Lena and Winnie) one, (Lena) seldom responded to acetyl choline and doses of as much as 5.0 mg. only produced a small and doubtful inhibition of urine flow /

flow (see Fig. 1, experiment of 10.11.48). The other 6 animals usually responded to doses of 1.0 to 3.0 mg., but of a total of 72 tests in 6 dogs only 42 (60 per cent.) produced a typical inhibition of urine flow of the type originally described.

Although quantitative comparison of the effects of similar doses of acetyl choline when given intravenously is not possible, because the amount of acetyl choline which reaches the hypothalamus will vary amongst other factors with the cardiac output and the level of serum cholinesterase, it was possible to make certain that some acetyl choline had reached the brain after every test by recording central effects such as licking of the lips and sighing respirations.

It has not been possible to reach any definite conclusion concerning the cause of the discrepancy between these results and those reported previously. The response was not more constant in those animals (Wolf, Winnie, Brownie and Buster) who had denervated kidneys, or in Blackie.

The rate of urine flow and the water load at the time of test do not appear to have any marked influence on the appearance or otherwise of an inhibition of urine flow due to acetyl choline although it /

it has occasionally been noticed that when acetyl choline has been ineffective early in a water diuresis, the same dose given later has produced a typical effect. This might be expected since it has been shown (Pickford, 1936) that the inhibitory effect of posterior pituitary extract varies indirectly with the water load.

The most likely factors which are responsible for the inconsistency of the response to acetyl choline are variations in diet and environment of the dogs, and their lack of regular training. In a small number of tests in this series (6) acetyl choline has apparently produced a diuretic response. In these, an increase of the rate of urine flow was apparent within 6 minutes after the injection and the urine flow rate was maintained above the preinjection level for 15 to 25 minutes. All these tests were performed on days when either the rate of water excretion was definitely abnormal or when the response to acetyl choline was not normal. Four of the tests were done on Lena.

Eserine salicylate.- In the doses used (1.0 to 2.0 mg. injected subcutaneously) eserine itself had usually no effect on the rate of urine flow, although it occasionally apparently produced a slow diminution of urine flow which was apparent about 20 minutes after the injection. During the period 20 minutes to 1 hour 30 minutes after eserine, not only were the duration /

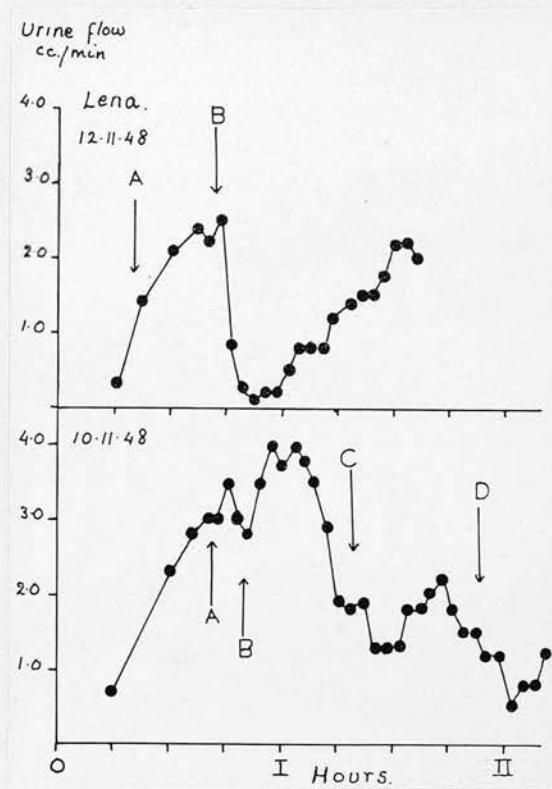


Fig. 1. To illustrate that eserine potentiates the effects of intravenous acetyl choline on the rate of urine flow.

Lower curve. At 0 hours 350 c.c. water p.o. and 2 mg. atropine sulphate s.c.

At A. 2 mg. Ach. I.V.

B. 3 mg. Ach. I.V.

C. 3 mg. Ach. I.V.

D. 5 mg. Ach. I.V.

Upper curve. At 0 hours 350 c.c. water p.o. and 2 mg. atropine sulphate s.c.

At A. 2 mg. eserine salicylate s.c.

B. 1 mg. Ach. I.V.

Abscissa - time in hours after test dose of water.

Ordinate - rate of urine flow in c.c./min.

p.o. - per oesophageal tube

s.c. - subcutaneously.

I.V. - intravenously.

duration and percentage inhibition of effective doses of acetyl choline increased, but doses which were previously ineffective became effective in evoking a response. This is shown clearly in Fig. 1.

The dog, "Lena", who was unreactive to intravenous acetyl choline alone, always reacted to doses of 0.5 to 1.0 mg. after eserine.

2. The Effect of Adrenaline on the Response to Acetyl Choline

In a number of experiments, tests were made of the effects produced on the rate of urine flow by a combination of adrenaline and acetyl choline. Both drugs were given intravenously to normal unanaesthetised atropinised dogs during a water diuresis, and also to Spot and Poppet at a time when their responses to acetyl choline were apparently normal.

The adrenaline (Parke Davis' solution with chloretone) was diluted with 0.9 per cent. saline 10 to 15 minutes before use so that the final volume injected was 0.5 c.c. Doses (5 to 30 µg.) were injected simultaneously with test doses of acetyl choline or at intervals (5 to 30 seconds) before the acetyl choline. When the drugs were given simultaneously they were mixed in the same syringe. When the adrenaline was given before the acetyl choline the two solutions were injected from separate syringes. /

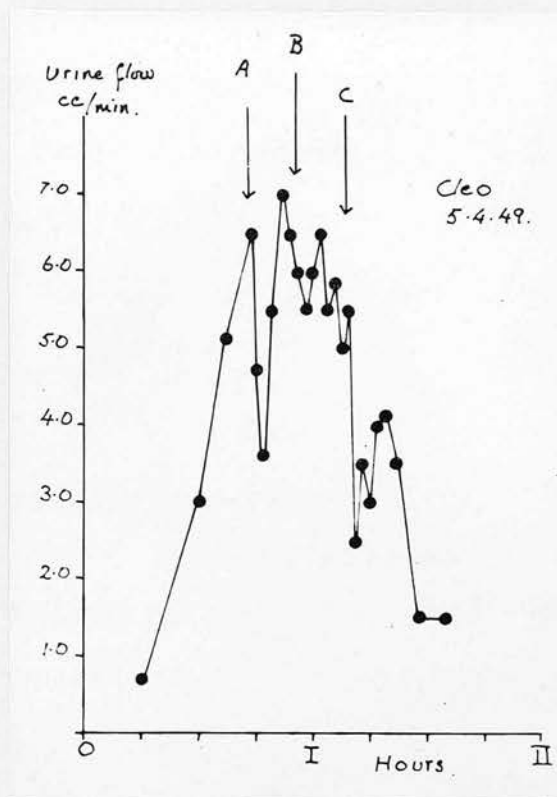


Fig. 2. To illustrate that adrenaline prevents the inhibition of urine flow caused by acetyl choline. At 0 hours 360 c.c. water p.o. and 2 mg. atropine sulphate s.c.

At A. 2 mg. Ach. I.V.

B.. 20 μ g. adrenaline I.V. 2 mg. Ach. I.V. 5 seconds later.

C. 2 mg. Ach. I.V.

Abscissa and Ordinate as in Fig. 1.

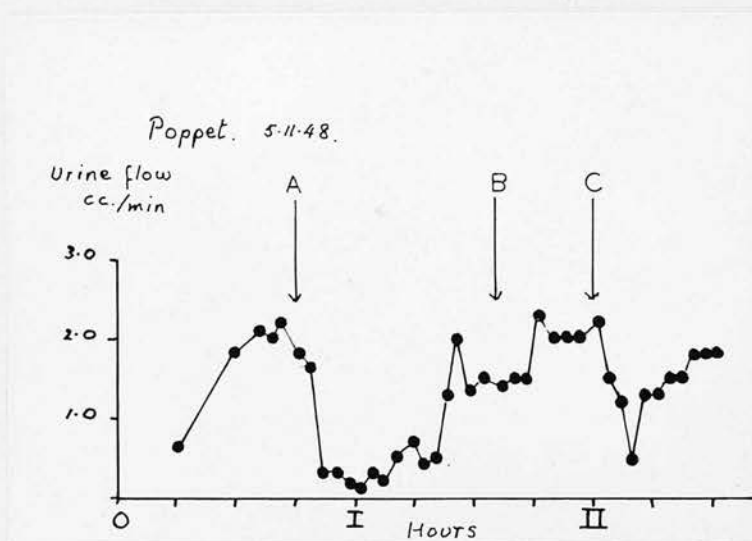


Fig. 3. To illustrate that adrenaline prevents the inhibition of urine flow caused by acetylcholine. At 0 hours 250 c.c. water p.c. and 1.0 mg. atropine sulphate s.c.

At A. 1 mg. Ach. I.V.

B. 20 µg. adrenaline I.V. 1 mg. Ach. I.V. 20 seconds later.

C. 1 mg. Ach. I.V.

Abscissa and Ordinate as in Fig. 1.

syringes. The first syringe was detached from the needle after the first injection and the second syringe was attached to the needle while it was still in the vein. The interval between the two injections was timed to the nearest second using a clock with a second hand.

The effect of adrenaline alone.- Adrenaline given in the doses described above either caused a transient inhibition of urine flow or had no noticeable effect. When an inhibition of urine flow occurred the maximum inhibition was seen within the first three minutes and the control rate of urine flow was attained in 3 to 8 minutes. This type of inhibition has been described before (O'Connor and Verney, 1945) and is ascribed to renal vasomotor changes.

Adrenaline combined with acetyl choline.- If adrenaline was injected simultaneously with or before acetyl choline the inhibitory response of acetyl choline was usually abolished, so that the rate of urine flow was either unchanged or showed only a transient diminution which might be ascribed to the presence of adrenaline (see Figs. 2 and 3). It has already been shown that under the conditions of this investigation the response to acetyl choline alone was variable so that it was necessary to assess the effects of the combination of drugs with great care. Where possible the combination of drugs was flanked by /

by two injections of acetyl choline alone, these, preferably, in the same water curve. Where that was not possible then it was necessary to demonstrate that acetyl choline was effective at least once in the same curve.

A total of 55 tests of the combination of adrenaline and acetyl choline have been made on five of the six dogs used for the tests of acetyl choline. Of these tests 30 produced no effect on the rate of urine flow while 25 (45 per cent.) had the typical inhibitory effect. There is no reason to suppose that acetyl choline by itself would have been ineffective in these tests, since the criteria described above had already been fulfilled. The largest number of tests of adrenaline and acetyl choline have been made on Cleo. In this dog 16 out of 25 injections of acetyl choline produced an inhibition of urine flow, but when adrenaline was given with or before the acetyl choline, only 5 out of 22 injections of acetyl choline were effective.

Unequivocal evidence of a suppression of the acetyl choline response by adrenaline has been obtained in each of the other 4 normal animals (Wolf, Brownie, Buster and Blackie) on many occasions, and also in Spot and Poppet at a time when their response to acetyl choline was apparently normal (see later).

At present there is insufficient evidence to make it /

it possible to decide why adrenaline is not always effective in preventing the inhibition of the rate of urine flow produced by acetyl choline. The presence or absence of a diminution of the rate of urine flow after the two drugs had been given in combination, appeared to be unrelated to the water load or to the rate of urine flow at the time of test, and the results were not more constant in the animals who had denervated kidneys or suprarenals than in normal animals. Indeed it was possible on more than one occasion to perform an identical experiment on the same dog on two separate days and to find that a dose of adrenaline which would effectively prevent the response to acetyl choline on one day would have no influence on the other day. The possibility exists that the results may be influenced in some way by the relationship between the size of the dose of the two drugs, acetyl choline and adrenaline, or the time interval between the injections. We have no information on that point.

A further observation, which may be of some importance, is that adrenaline has always failed to influence the response to acetyl choline when both drugs have been given in combination after eserine. Twelve such tests have been made on Lena, using various doses of adrenaline and acetyl choline with negative results. Similar responses have also been observed with Cleo, Blackie and Brownie.

3. Chronic Experiments in Dogs Recovering from the Effects of D.F.P. (diisopropyl fluorophosphate) Injected into the Supraoptic Nucleus

The four dogs (Spot, Poppet, Snapper and Trix) used in this investigation had all had D.F.P. (diisopropyl fluorophosphate) injected into both supraoptic nuclei 14 to 37 days before these observations were begun. The total dose of D.F.P. given to Spot, Snapper and Trix was 120 $\mu\text{g.}$, it was injected in 3 doses (40 $\mu\text{g.}$ in 0.002 c.c.) into the right and left supraoptic nuclei. Poppet had a total of 160 $\mu\text{g.}$ into both supraoptic nuclei in four doses.

When the investigations now reported were begun the water exchange, measured in a metabolism cage as fluid intake and urine output, was normal. Measurements of the water metabolism of Spot and Poppet were also made for two periods of 10 days in the middle of the investigations and gave results which were similar to those obtained preoperatively. In addition the water intake of Snapper and Trix was recorded for the first 2 months after operation and it was found to vary only within the normal range. Since the polyuria of diabetes insipidus is almost invariably associated with a polydipsia, it seems reasonable to suppose that these animals were also not polyuric.

Spot was killed 33 weeks after operation and Poppet after 27 weeks. The other animals (Snapper and /

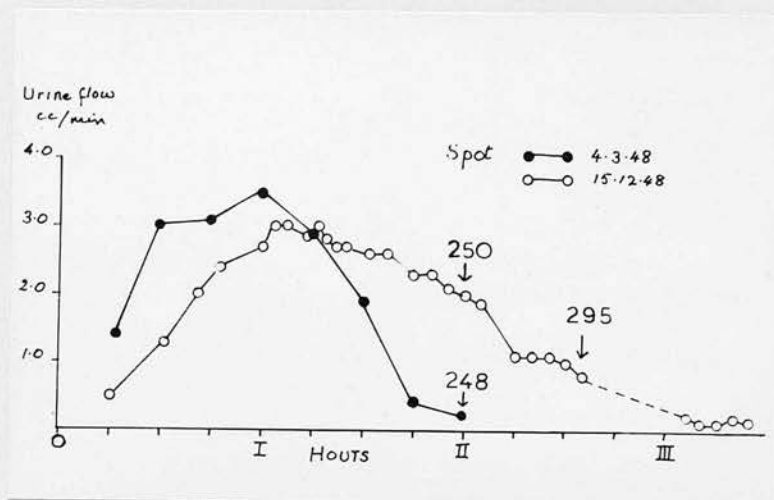


Fig. 4.

To demonstrate the variation in water excretion before and after the injection of D.F.P. into the supraoptic nuclei. 250 c.c. of water were given p.o. at 0 hours in each of the 2 curves shown.

Preoperatively, (black circles) all the extra water injected was excreted in 2 hours and the rate of urine flow had returned to the resting level.

Postoperatively, (open circles) although the extra water had been excreted in 2 hours the resting rate of urine flow was not attained in $2\frac{1}{2}$ hours.

The figures in the graph show the total volume of urine excreted from the beginning of the experiment at the time indicated.

Abscissa - time in hours after test dose of water.

Ordinate - rate of urine flow in c.c./min.

p.o. - per oesophageal tube.

and Trix) are still surviving 21 and 19 weeks after operation respectively. Serial sections were cut of the hypothalamic and pituitary regions of Spot and Poppet. The results of the histological examination of these are reported in the appendix.

Water diuresis.- All four dogs showed a good diuretic response to water. Trix, Poppet and Snapper excreted the total dose of water (250 c.c.) within two and a half hours, when the urine flow rate returned to the normal resting level. Spot, on the other hand, usually excreted 20 to 50 c.c. more water than she was given in two to two and a half hours and even then the resting rate of urine secretion was usually not attained. Fig. 4 shows pre- and postoperative responses of this dog to the administration of 250 c.c. water.

Response to acetyl choline.- Intravenous injections of acetyl choline (dose 0.1 to 2.0 mg. in 0.5 c.c. 0.9 per cent. saline) have been given to these animals during water diuresis at frequent intervals after operation. The animals were all given atropine (0.75 to 1.0 mg. subcutaneously) 20 to 45 minutes before the acetyl choline.

The response to acetyl choline was found to be extremely variable for the first 3 to 4 months after operation; thereafter a slow recovery occurred and the typical inhibition of urine flow was subsequently produced.

TABLE I

Type of Response to 1.0 mg. Acetyl Choline

(a) Poppet

Date	↓	↑	-	?
9. 6.48		1		
10. 6.48		11		
12. 6.48	1		1	
14. 6.48		1	1	
15. 6.48	1	1		
16. 6.48				1
24. 6.48	1		1	
18. 8.48		1		
24. 8.48	1			
2. 9.48	11			
3. 9.48	1			
27.10.48	11			
5.11.48	11			
15.11.48	1			
17.11.48	1			
23.11.48			1	

89th post-operative day

(b) Spot

Date	↓	↑	-	?
4. 5.48				1
10. 5.48	1			
14. 5.48			1	
10. 6.48	1			
16. 6.48	1			
21. 6.48		11		
22. 6.48	11			
25. 8.48			11	
26. 8.48				1
30. 8.48			1	
31. 8.48	1			
27.10.48	1			
5.11.48	11			
13.11.48	11			
24.11.48	11			
3.12.48	11			

146th post-operative day

↓ = inhibition of urine flow rate

↑ = increase of urine flow rate

- = no effect on the rate of urine flow

? = results equivocal

TABLE I (Contd.)

(c) Snapper

Date	↓	↑	-	?
11.1.49			11	
10.1.49			1	
14.1.49			1	
17.1.49			1	
26.1.49			1	
7.2.49			1	
28.2.49			1	
23.3.49			1	
14.3.49	1			
28.3.49	1			
1.4.49	1			
14.4.49	1			

103rd post-operative day

(d) Trix

Date	↓	↑	-	?
7.1.49			1	
10.1.49				1
14.1.49			1	
28.1.49			1	
9.2.49			1	
25.3.49	1			
28.3.49			1	
17.3.49		1		
29.3.49	1			
4.4.49	1			

104th post-operative day

↓ = inhibition of urine flow rate

↑ = increase of urine flow rate

- = no effect on the rate of urine flow

? = results equivocal

Table Ia shows the type of response obtained after intravenous injection of 1.0 mg. acetyl choline in Poppet. The observations began on the 14th post-operative day and from then until the 89th post-operative day, no consistent results were obtained. Of 13 injections on 8 separate days, a normal anti-diuresis was only obtained 3 times. 6 tests resulted in an apparent diuresis and 3 others showed no effect on the rate of urine flow. It should be noted that 5 of the diuretic responses occurred within the first 20 days after operation. After the 89th day, 10 tests out of a total of 11 tests on 18 separate days produced a typical antidiuresis.

Table Ib shows similar responses in Spot. In this animal observations began on the 27th post-operative day; from this day until the 146th day 13 tests were made with 1.0 mg. acetyl choline. The rate of urine flow was decreased as a result of the injection on 5 occasions, 2 tests gave inconclusive results and 4 were apparently without effect. A diuresis was only observed twice, both times on the 75th day. After the 146th day, 10 out of 10 injections of acetyl choline produced the typical diminution of urine flow rate.

Figs. 5 and 6 show diuretic responses to acetyl choline in Spot and Poppet. These responses showed the following characteristics; the maximum rate of urine /

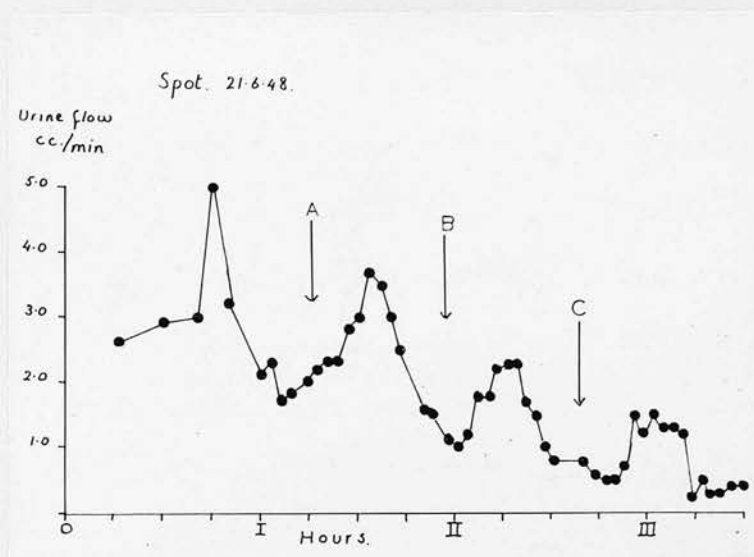


Fig. 5. To illustrate the diuretic effect of acetyl choline in dogs recovering from the effects of D.F.P. injected in the supraoptic nucleus At 0 hours 250 c.c. water p.o. and 0.75 mg. atropine sulphate s.c.

At A. 1 mg. Ach. I.V.

B. 1 mg. Ach. + 5.0 μ g. adrenaline I.V.

C. 1 mg. Ach. I.V.

Abscissa and Ordinate as in Fig. 1.

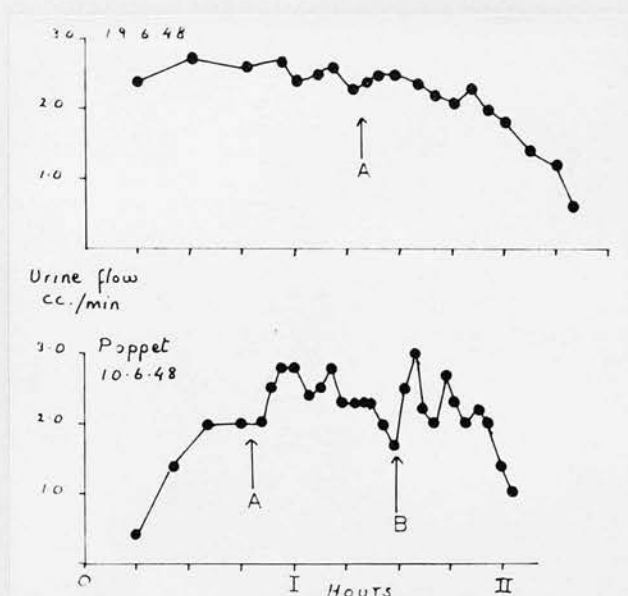


Fig. 6. The diuretic effect of acetyl choline in dogs recovering from the effects of D.F.P. injected into the supraoptic nucleus.

Lower curve. At 0 hours 250 c.c. water p.o. and 0.75 mg. atropine sulphate s.c.

At A. 1 mg. Ach. I.V.

B. 1 mg. Ach. I.V.

Upper curve. Water diuresis curve - 250 c.c. water p.o. at 0 hours.

At A. 0.5 c.c. saline I.V.

(Because the hydrating dose of water had been given only 1 hour before the start of the experiment the initial rate of urine flow is high).

Abscissa and Ordinate as in Fig. 1.

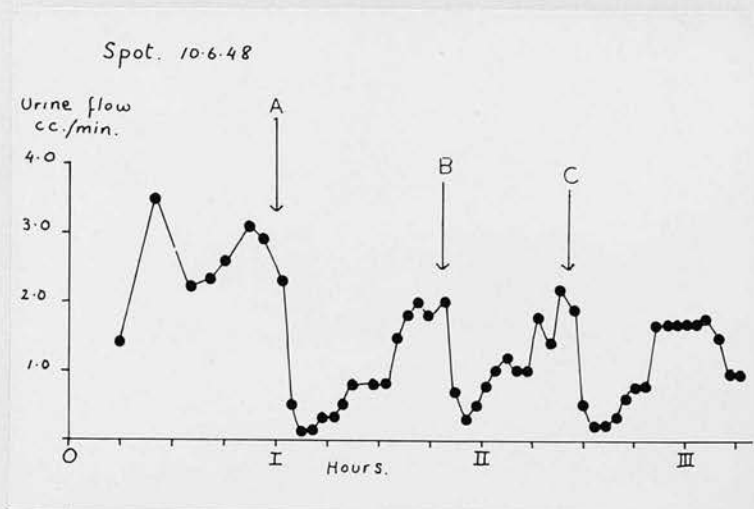


Fig. 7. An antidiuretic effect of acetyl choline in a dog recovering from the effects of D.F.P. injected into the supraoptic nucleus. At 0 hours 225 c.c. water p.o. and 0.75 mg. atropine s.c.

At A. 1 mg. Ach. I.V.

B. 1 mg. Ach. + 10 µg. adrenaline I.V.

C. 1 mg. Ach. + 20 µg. adrenaline I.V.

Abscissa and Ordinate as in Fig. 1.

urine flow was attained 6 to 10 minutes after the injection and the urine flow rate was maintained above the preinjection level for 20 to 30 minutes; increases of 40 to 80 per cent. were observed.

There was no relationship between the rate of urine flow or the water load at the time of the test and the type of response produced by acetyl choline. Indeed it was found that all doses of acetyl choline from 0.1 to 2.0 mg. tended to produce the same type of response on the same day. An idea of the variability of the response can be derived from a comparison of Figs. 5 and 7 which show the effects produced by 1.0 mg. acetyl choline on Spot on two separate days.

Similar results have been obtained with Snapper and Trix, although the observations have not been continued long enough on these dogs for full recovery to have occurred. Table Ic and d show the type of response obtained as a result of the injection of 1.0 mg. acetyl choline in Snapper and Trix. In Snapper acetyl choline had no effect when given from the 37th postoperative day to the 103rd day. After that day 1.0 mg. apparently produced a slow inhibition of the rate of urine flow which was not typical of the normal acetyl choline response. Trix also was unresponsive to acetyl choline but showed some signs of recovery on the 104th postoperative day. Other doses of acetyl /

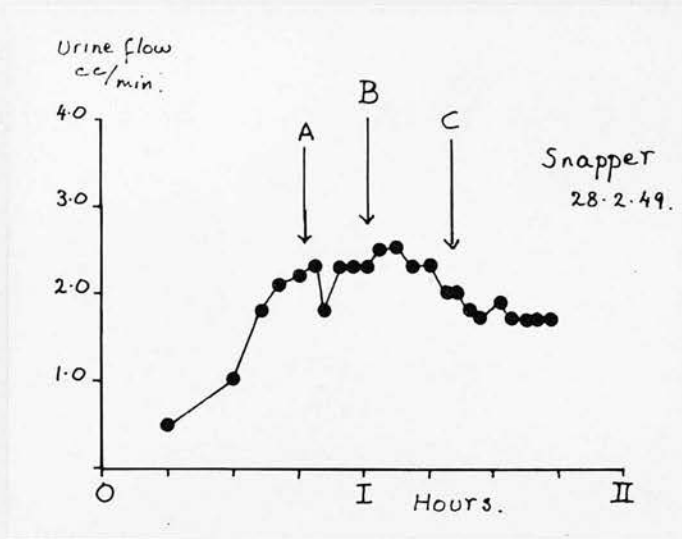


Fig. 8.

To demonstrate that the effect of acetyl choline on the rate of urine flow is not potentiated by eserine in dogs recovering from the effects of D.F.P. injected into the supraoptic nucleus.
At 0 hours 250 c.c. water p.o. and 1.0 mg. atropine sulphate s.c.

At A. 1 mg. Ach. I.V.

B. 1 mg. eserine salicylate s.c.

C. 1 mg. Ach. I.V.

Abscissa and Ordinate as in Fig. 1.

acetyl choline (1.0 to 2.0 mg.) were without effect during the early period.

The effects of eserine.- Eserine salicylate (dose 1.0 mg. subcutaneously) completely failed to potentiate a response to 1.0 mg. acetyl choline in Snapper and Trix, when the test was made during the period that acetyl choline given alone was ineffective (see Fig. 8).

The effects of adrenaline on the response to acetyl choline.- Injections of adrenaline did not vary the response to acetyl choline whether diuretic or antidiuretic, in Spot and Poppet (see Figs. 5 and 7) during the period when the response to acetyl choline was abnormal. Insufficient work has been done on this to make this a dogmatic statement. During the period when the acetyl choline responses were normal adrenaline was also effective in inhibiting the diminution of urine flow produced by acetyl choline (see Fig. 3).

4. Morphine /

TABLE II

The Effects of Morphine Injected into the Supraoptic Nucleus

No. of Expt.	Dose of Morphine µg.	Time from Injection to Maximum Inhibition of Urine Flow (minutes)	% Inhibition of Urine Flow (maximum)	Time from Injection for Urine Flow Rate to reach 50% Recovery
M 50	160	30	98	No recovery in 4 hours
M 51	32	19	87	1 hr. 9 min.
M 52	16	16	90	1 hr. 4 min.
M 53	32	20	89	2 hr. 30 min.
M 54	8	17	88	48 min.
M 55	4	11	55	Only slight recovery because most of ingested water already excreted
M 60	4	10	40	do.

Note: Each experiment represents a different animal

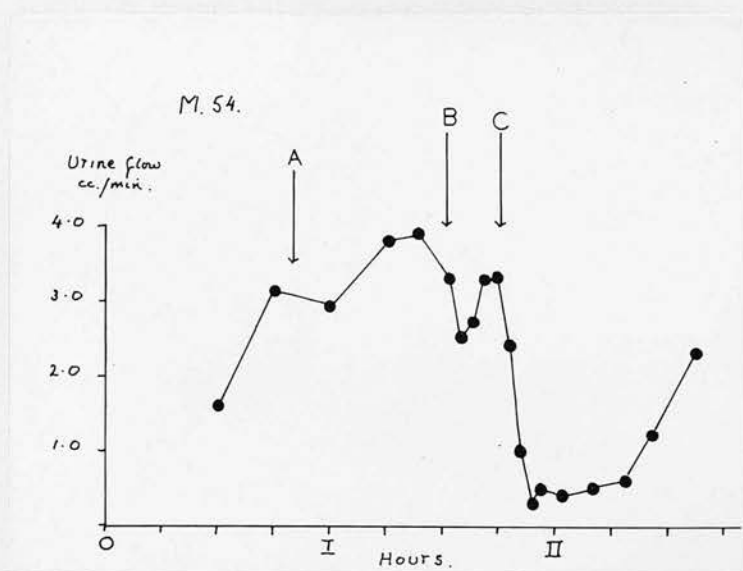


Fig. 9.

The effect of morphine injected into the supraoptic nucleus.

At 0 hours 2.4 g. chloralose in 320 c.c. water p.o.

At A. 0.15 g. chloralose in 20 c.c. water I.V.

B. Attempt at injecting 8 μ g. morphine sulphate into the supraoptic nucleus.

C. 8 μ g. morphine sulphate in 0.002 c.c. saline injected into the supraoptic nucleus.

Abscissa and Ordinate as in Fig. 1.

4. Morphine

(1) The Effects of Morphine Injected into the Supra-optic Nucleus of Dogs under Chloralose Anaesthesia and During Water Diuresis

Various doses of morphine have been injected into the supraoptic nucleus in animals under chloralose anaesthesia and during water diuresis. In the dose and concentrations used (160 μ g. to 4.0 μ g. in 0.002 c.c. normal saline), morphine always produced an inhibition of the rate of urine flow (see Table II and Fig. 9). The diminution of urine flow was abrupt in onset and the maximum inhibition was usually reached in 10 to 20 minutes; thereafter the rate of urine flow gradually increased and 50 per cent. recovery of the control rate of urine excretion was reached in 40 minutes to two and a half hours. Table II shows that there was some degree of relationship between the dose of morphine and the magnitude of the response and, in general, the larger doses of morphine produced a more complete inhibition of urine flow from which recovery was delayed longer than did the smaller doses.

Records of the blood pressure were made in two experiments before, during and after the injections. These did not show any variation as a result of the operative interference. Fig. 10 is a record of the blood pressure from the experiment shown in Fig. 9.

Measurements /

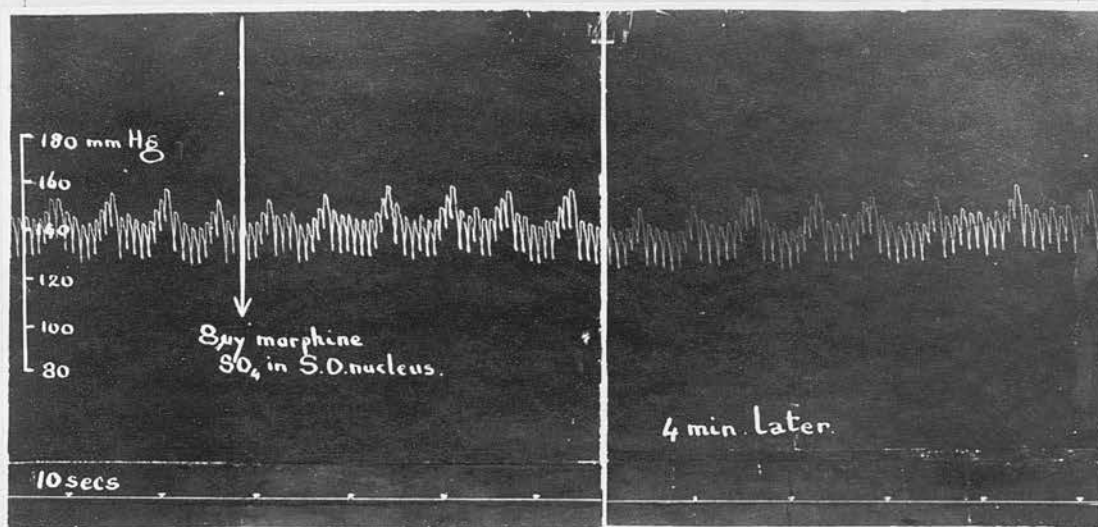


Fig. 10. Blood pressure tracing obtained from the femoral artery of the dog used in the experiment shown in Fig. 9.

- (a) Shows the level of the blood pressure before, and immediately after, the injection of 8 μ g. morphine sulphate into the supraoptic nucleus.
- (b) 4 minutes after (a). The blood pressure has not shown any variation as a result of the injection.

Measurements of the diodone and creatinine clearance have been made at intervals in each experiment. With the large dose of morphine (160 μ g.) there was a marked and persistent depression of both clearances. With the other doses (4 to 32 μ g.) the changes in clearance were inconsistent and unrelated to the changes in urine flow rate. The creatinine clearance was unchanged by the morphine in 4 experiments. In one experiment it showed an increase which was maintained throughout the period of maximum inhibition of urine flow and in one other experiment there was a temporary depression of creatinine clearance. The diodone clearances remained unchanged throughout the period of maximum inhibition of urine flow in four experiments, although in three of these there was a marked increase in clearance during the recovery period. An increase and a decrease in diodone clearance was each seen once after morphine.

These results were independent of the use of morphine sulphate or morphine hydrochloride.

(2) The Effects of Morphine Injected Intravenously into Unanaesthetised Dogs During Water Diuresis

The intravenous injection of 0.25 to 1.0 mg. of morphine sulphate or morphine hydrochloride into unanaesthetised dogs during water diuresis produced an inhibition of the rate of urine flow. The diminution of urine flow was abrupt in onset and reached a maximum /

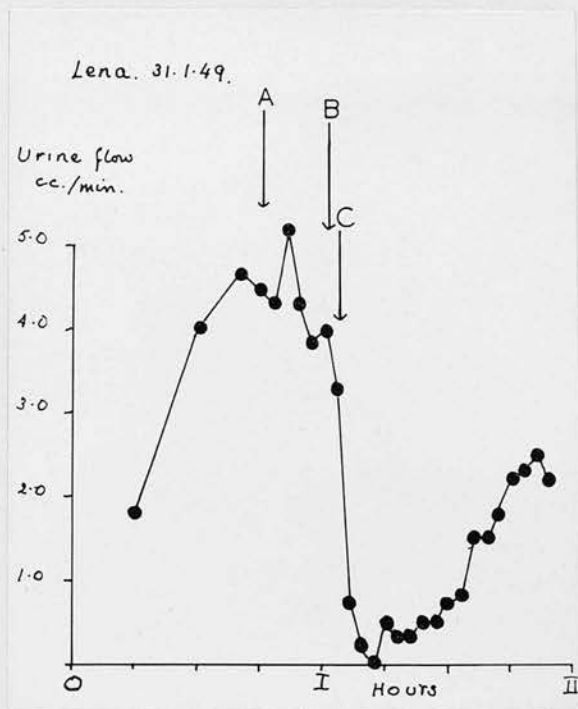


Fig. 11.

The antidiuretic effect of morphine in the normal unanaesthetised dog.

At 0 hours 350 c.c. water p.o. and 2.0 mg. atropine sulphate s.c.

At A. 0.25 mg. morphine sulphate IV.

B. 0.5 mg. morphine sulphate I.V.

C. The dog was observed to retch but did not actually vomit.

Abscissa and Ordinate as in Fig. 1.

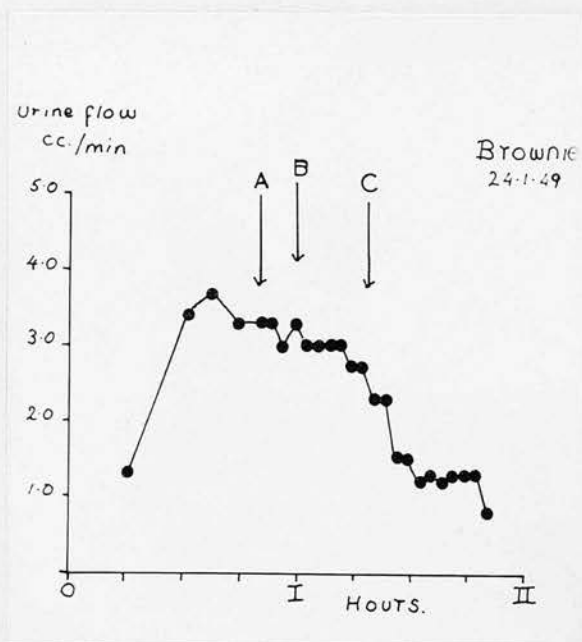


Fig. 12.

The effect of eserine on the response to morphine.

At 0 hours 250 c.c. water p.o. and 1.0 mg. atropine sulphate s.c.

At A. 0.125 mg. morphine sulphate I.V.

B. 1.0^{mg.} eserine salicylate s.c.

C. 0.125 mg. morphine sulphate I.V.

Abscissa and Ordinate as in Fig. 1.

maximum within 15 minutes; thereafter a slow recovery occurred. This result was obtained in animals in which the kidneys were innervated or denervated and also in one (Blackie) after denervation of the suprarenals. The response to intravenous morphine was also unaltered by the administration of atropine to the animal.

Considerable individual variation was, however, found in the response and there was some difficulty in finding a dose of morphine which would be effective in causing an antidiuresis without, at the same time, causing vomiting. Of the four animals used in this investigation, two (Lena and Brownie) always vomited if the dose of morphine injected was sufficient to cause an antidiuresis. A typical inhibition of urine flow rate could be produced in Blackie and Winnie by the intravenous injection of 0.5 to 1.0 mg. morphine without causing any objective symptoms beyond some licking of the lips. Fig. 11 shows a response to 0.5 mg. morphine sulphate in Lena.

The subcutaneous administration of eserine salicylate did not appear to potentiate the response to morphine in a comparable way to that to acetyl choline. Fig. 12 shows an experiment on a dog (Brownie) in whom 0.25 mg. morphine sulphate injected intravenously produced a profound depression of the rate of urine flow, but caused vomiting. 0.125 mg. morphine intravenously had /

had no effect on the rate of urine flow and the response was not potentiated by eserine.

(3) The Effects of Morphine Injected Intravenously during Water Diuresis into Dogs Recovering from the Effects of D.F.P. Injected into the Supraoptic Nucleus

The intravenous injection of morphine (dose 0.5 to 1.0 mg.) into dogs who had had D.F.P. injected into the supraoptic nucleus, also led to an inhibition of urine flow. This result was obtained even at such a time after the operation when acetyl choline was not effective in causing any change in the rate of urine flow, or caused a diuresis.

IV. SUMMARY OF EXPERIMENTAL FINDINGS

1. In the unanaesthetised atropinised dog, intravenous injections of acetyl choline during a water diuresis produce an inhibition of urine flow of the type described by Pickford (1939). The results in this series of experiments have not been so constant as those originally described, and possible reasons for this have been discussed.

The effect of acetyl choline on the rate of urine flow is potentiated by eserine.

2. Intravenous injections of adrenaline simultaneously or at various (5 to 30 seconds) intervals before test doses of acetyl choline are usually effective in preventing the typical antidiuretic effect of acetyl choline.

3. Chronic experiments performed on dogs recovering from the effects of D.F.P. injected into the supraoptic nucleus have shown that, although the acute effects of D.F.P. are recovered from in about 14 days, full recovery does not occur for some 3 to 4 months. During this time there is an abnormal response to intravenous injections of acetyl choline.

4. Morphine injected into the supraoptic nucleus produces an antidiuresis of the "pituitary type". This response is not related to changes in renal blood flow.

Normal dogs, whether atropinised or not, react to intravenous injections of morphine by an anti-diuresis. Effective doses, however, nearly always cause vomiting.

Similar effects, were found in dogs recovering from the effects of D.F.P. injected into the supra-optic nucleus.

V. DISCUSSION

The experiments now reported were planned to provide information on two points. Firstly, it was hoped to see if adrenaline would affect the inhibition of urine flow produced by acetyl choline in the atropinised dog in a similar way to that described by Rydin and Verney (1938) and O'Connor and Verney (1945) for the inhibition of water diuresis by emotional stress. The second object of the experiments was to investigate the antidiuretic properties of morphine (de Bodo, 1944) and to obtain more precise information as to the site and mode of action of this drug. Later it became possible to study the effects of acetyl choline and morphine in dogs recovering from the effects of D.F.P. injected into the supraoptic nucleus.

The experimental findings will first be discussed under three separate headings:-

Adrenaline and Acetyl Choline

These observations have shown that, under certain conditions, the inhibition of urine flow that is produced by intravenous injections of acetyl choline in the atropinised dog can be prevented by adrenaline. This finding is analogous to that of Verney and his co-workers who found that the inhibition of urine flow due to emotional stress could also be prevented by adrenaline, and it provides further evidence of the similarity /

similarity between the antidiuretic response to emotional stimuli and that induced by acetyl choline. It has already been shown by Pickford (1939) and Verney (1947) that the inhibitory responses to intravenous acetyl choline and to emotional stress show the same time relationships. Both responses are independent of changes in blood pressure, increase the rate of chloride excretion and are reduced after removal of the neural lobe of the pituitary gland. There can be little doubt that intravenous injections of acetyl choline are followed by an increased output of antidiuretic hormone.

There is some evidence that acetyl choline may be concerned with the transmission of the nervous impulse in the supraoptic nucleus, because the injection of acetyl choline into this region produces an inhibition of urine flow (Pickford, 1947). This evidence, however, does not exclude the possibility that acetyl choline may also stimulate other neurones which may be concerned with the ultimate release of the antidiuretic hormone.

Verney (1947) has studied, in some detail, the mechanism of the action of adrenaline in the inhibition of urine flow due to emotional stress. To summarise his findings:- The effect of adrenaline is probably not on the kidneys because it does not inhibit the response to the injection of posterior pituitary extract; /

extract; and the effect is probably not caused through a rise in blood pressure because equal increases in blood pressure produced by occlusion of the carotid arteries to those induced by the doses of adrenaline he employed were not effective in preventing the antidiuresis of emotional stress. The effects of adrenaline are not specific, however, and tyramine is also active in this respect.

It would appear likely that adrenaline and tyramine have central actions preventing the release of the antidiuretic hormone; but whether these are exerted directly on the neurones responsible for the ultimate release of the antidiuretic hormone or on other structures cannot be determined at present.

A similar antagonism between the effects of adrenaline and acetyl choline has been observed in many parts of the nervous system (see review by Burn, 1945). It has generally been considered that small doses of adrenaline potentiate the effects of acetyl choline in nervous and ganglionic transmission and that large doses are depressant. We have not observed any potentiation of acetyl choline by adrenaline, but this may only be due to the difficulties already discussed (see page 27) in obtaining quantitative measurements under the conditions of our experiments.

At /

At present we have no information concerning the reason for the failure of adrenaline to inhibit the response to acetyl choline in every test. It is probable that the size of the dose of the two drugs and the time interval between the two injections may determine the results in some degree. These relationships would not be easy to investigate using the technique that has been employed up to date, and it is hoped to get more information on this point from the results of injections of the two drugs into the carotid artery. It is of interest, however, that adrenaline is not always effective in preventing the antidiuresis of emotional stress, and in Rydin and Verney's (1938) original experiments only three out of four dogs showed the effect.

The absence of any effect of adrenaline on the response to acetyl choline in the eserinated animal is interesting, but the reason for this is indefinite. Possibly this finding may indicate that the size of the dose of acetyl choline that reaches the supraoptic nucleus determines the appearance, or otherwise, of an antidiuretic response after adrenaline and acetyl choline have been given in combination.

Diisopropyl /

Diisopropyl Fluorophosphate

In view of the possibility that acetyl choline is concerned with the transmission of the nervous impulse in the supraoptic nucleus it became of interest to see what would be the effect of injecting D.F.P. into this region.

The acute effects produced by the injection of D.F.P. into the supraoptic nucleus have already been described (Pickford, 1948). It has been suggested that both the initial inhibition of urine flow which lasts for some hours after the injection, and the polyuria which occurs at the end of the inhibitory period and lasts for several (2 to 16) days could be explained on the basis of the anticholinesterase action of D.F.P. (Mackworth, 1942). The antidiuresis would thus be caused by a stimulant action of the naturally formed acetyl choline within the nucleus leading to an increased output of antidiuretic hormone, and the polyuria by a paralytic effect of higher concentrations of acetyl choline. The question as to whether the injections of D.F.P. produce their effects by trauma to the supraoptic nucleus must also be considered, because transient polyuria occurring after injury to the hypothalamic region is a common occurrence (Pickford and Ritchie, 1945). We have some information on this point. On one occasion a dog was allowed to recover after the injection of morphine into the /

the supraoptic nucleus on both sides. This dog did not suffer from an increased fluid exchange during the five postoperative days for which it was observed. One other dog was killed three days after the injection of D.F.P. into one supraoptic nucleus. This dog did not exhibit polydipsia in the postoperative period and histological examination did not reveal any changes in the supraoptic or other hypothalamic nuclei other than a needle track into the supraoptic nucleus on one side. Therefore, unless it is assumed that traumatic effects of D.F.P. could be exerted in the absence of any histological changes, the observed effects cannot be attributed to trauma alone.

It is not easy to attribute the uncertain reactions to acetyl choline that occur in the first three to four months after the polyuric phase solely to an inhibition of the cholinesterase in the supraoptic nucleus. In the first two dogs to be studied (Spot and Poppet) this period was characterised by an extreme variability of the response to acetyl choline. The other two animals did not respond to acetyl choline during the comparable period, and no potentiation of the response could be obtained by eserine. We know nothing of the state of the supraoptic nucleus during this period, but both Spot and Poppet had less than the normal number of cells in the supraoptic nucleus even when they were apparently fully recovered. Spot had /

had approximately 50 per cent. and Poppet 70 per cent. of the normal number of cells. Further experiments are being carried out in which animals will be killed at various intervals after the injection of D.F.P. into the nucleus, and the hypothalamus will be examined histologically. It is unlikely, however, that at any time after the initial polyuria were less than 15 per cent. of the supraoptic nuclear cells functioning, because the animals did not show an increased fluid exchange (Fisher et al., 1938). That the animals may have had some abnormality of water balance is shown by the abnormal response of Spot to water.

One is reminded of the fact that approximately 70 per cent. of the cells of the supraoptic nucleus degenerate after removal of the posterior lobe of the pituitary gland in the dog (O'Connor, 1946). This operation is associated with a 95 per cent. decrease of the antidiuretic effects of emotional stimuli (Verney, 1947), but diabetes insipidus is usually not produced and the dogs respond to water in a normal manner (Pickford and Ritchie, 1945). One is led to conclude that, after degeneration and inactivation of a certain proportion of the cells in the supraoptic nuclei, the posterior pituitary is unable to discharge antidiuretic hormone rapidly enough to cause an inhibition of water diuresis but it can manufacture sufficient to keep the animals from being polyuric.

It has been shown (Mazur and Bodansky, 1946) that the recovery of brain cholinesterase following intramuscular injection of D.F.P. into rabbits is not complete in 50 days, but that 20 per cent. of the normal activity is attained in 10 days, which is the level at which the animals are symptomless. This latter period corresponds closely to the period of diuresis in our animals. There are thus three possible explanations for the period of abnormal responses that we have observed. Either that the cholinesterase is not regenerated so rapidly in our animals as in those of Mazur and Bodansky, or that subnormal cholinesterase levels, while causing no symptoms in the intact animal, may interfere with the passage of nervous impulses in some way, or that D.F.P. has some other effect than that which can be ascribed to its anticholinesterase activity.

Further explanations are required of the diuretic responses to acetyl choline. This effect was observed in two dogs only (Spot and Poppet) and was a relatively constant phenomenon only in Poppet. It is not considered likely that changes in blood pressure or renal blood flow could be solely responsible for the increase in urine flow rate because of the time relationship between the injection of acetyl choline and the response. If the diuresis is to be explained by a paralytic /

paralytic effect of acetyl choline accumulating in the absence of cholinesterase then it would be expected that the diuresis would occur more readily after large doses of acetyl choline or that eserine would potentiate the appearance of the response. This has not been the case in this investigation. A diuretic response might be obtained if the normal supraoptic innervation of the neurohypophysis was inactive and acetyl choline stimulated the production of a diuretic hormone, possibly from the adenohypophysis. We have occasionally observed apparently similar diuretic responses to the injection of acetyl choline in normal animals, but these responses were always obtained in dogs when the rate of water excretion was abnormal. The finding that the number of cells containing Nissl granules in the supraoptic nucleus of Poppet was decreased (see appendix) may be correlated in some way with the greater number of apparent diuretic responses to acetyl choline that were obtained in this animal. This result requires further confirmation, however.

It is, therefore, clear that, in the absence of further work, no precise explanation can be offered for the more chronic effects produced by the injection of D.F.P. into the supraoptic nucleus. The initial effects, shown as changes in the water balance, are not inconsistent with the anticholinesterase action of D.F.P.

Morphine /

Morphine

It will be recalled (see page 16) that de Bodo (1944) found that morphine given to dogs by intramuscular or intravenous injection decreased the amount of water excreted in three hours after a test dose of water and that this effect was not seen in animals suffering from diabetes insipidus. He, therefore, assumed that morphine had a direct stimulant action in the neurohypophysis leading to an increased output of antidiuretic hormone.

The experiments of the present series lend some support to this view. It has been shown that intravenous injection of morphine into unanaesthetised dogs during water diuresis produces an inhibition of urine flow by the "pituitary type". The appearance of this inhibition is unaffected by denervation of the kidneys and the suprarenal glands, and it can be obtained in the atropinised animal. Further, direct injection of morphine into the supraoptic nucleus of the chloralised dog also produces a "pituitary type" of inhibition of urine flow. This latter effect is exerted independently of changes in systemic blood pressure, renal plasma flow or glomerular filtration rate.

In our experiments, as in those of de Bodo, the morphine was always given at least 45 minutes after the water, so that the effects cannot be ascribed to an /

an inhibition of water absorption from the intestine.

Morphine has been shown to have an anticholinesterase action in vitro (Bernheim and Bernheim, 1936; Torda and Wolf, 1947), and it has been suggested that certain of its effects in the whole animal could be explained by such an action (Slaughter and Gross, 1940). Hebb and Konzett (1949, personal communication) were not in favour of such a simple explanation for the action of morphine on the perfused superior cervical ganglion of the cat.

We do not feel justified in attributing our results on the normal unanaesthetised dog to an anticholinesterase action of morphine, or to any other direct action of morphine on the supraoptic nucleus, because of the simultaneous occurrence of vomiting, or obvious nausea, with the appearance of the diminution of the rate of urine flow after many of the injections. It has frequently been observed that vomiting due to other causes can bring about an inhibition of urine flow of the "pituitary type" during water diuresis (Pickford, personal communication), and it is suggested that in nausea the supraoptic nucleus may be stimulated possibly from cortical levels. It is impossible /

impossible to exclude such an effect even in those instances when morphine has apparently produced an inhibition of urine flow without causing any objective symptoms. De Bodo (1944) did not report the occurrence of nausea in his animals, but since the dose of morphine he employed was 10 to 100 times as large as that used in the present investigation it seems unlikely that he escaped this complication.

When morphine is injected directly into the supraoptic nucleus it can apparently directly stimulate the release of the antidiuretic hormone from the neural lobe.

One anomalous action of morphine has still to be explained. In dogs that are recovering from the effects of D.F.P. injected into the supraoptic nucleus intravenous injections of morphine cause an antidiuresis even at such a time when injections of acetylcholine are without effect or cause a diuresis. If the previous theory is correct, and the inhibition of urine flow produced by morphine in the unanaesthetised dog is connected with the nausea or vomiting that accompany the injection, then a possible explanation of the action of morphine in dogs after D.F.P. is that D.F.P. prevents in some way the action of injected acetylcholine on the nucleus but that natural stimuli are still effective. Experiments are in progress to see if dogs will react to emotional stimuli after the injection of D.F.P. into the supraoptic nucleus.

VI. CONCLUSION

Variations in the amount of antidiuretic hormone secreted from the neurohypophysis are reflected as changes in the rate of urine flow from the kidneys. The evidence has already been discussed in the introduction to this thesis for the belief that the neurohypophysis is innervated from cells in the supraoptic nucleus; and that the integrity of the nervous connections between the supraoptic nucleus and the neurohypophysis is essential for the normal control of the rate of urine secretion.

Stimuli applied to the supraoptic nucleus, either directly, or indirectly, cause changes in the rate of urine excretion; thus these changes can be used to indicate the state of activity of the supraoptic nuclear cells.

The experiments reported in this thesis have lent further support to the view that acetyl choline stimulates the cells of the supraoptic nucleus. The results also give rise to many interesting speculations, the exact origin of which is obscure at present.

The supraoptic nucleus is a comparatively easily accessible central synapse, and in recording variations of the rate of urine secretion a reliable index of the state of activity of the cells is present.

It /

It is likely that further work on these lines may provide important information as to the behaviour of central synapses.

VII. APPENDIX

The Animals and their Operation Histories

Normal Dogs

74. Blackie: Black Spaniel ♀

- 1.6.48. Weight, 17.7 kg. Perineotomy
18.6.48. Weight, 15.4 kg. Right suprarenal removed.
13.9.48. Denervation of left suprarenal.
21.1.49. Weight, 21.0 kg.
9.2.49. Killed.
No sign of right suprarenal at post-mortem examination.

77. Winnie: Collie ♀

- 14.6.48. Weight, 7.3 kg. Perineotomy.
30.7.48. Developed cellulitis and gangrene of leg. Killed.

79. Lena: Dalmatian ♀

- 20.8.48. Weight, 11.8 kg. Perineotomy.
30.8.48. Developed distemper. Treated with serum.
10.9.48. Weight, 11.3 kg.
21.1.49. Weight, 11.8 kg.
17.3.49. Weight, 13.0 kg.

80. /

80. Cleo: Bull Terrier ♀

6.9.48. Perineotomy

21.1.49. Weight, 16.0 kg.

17.3.49. Weight, 16.8 kg.

81. Brownie: Smooth Haired Brown Terrier ♀

13.10.48. Weight, 11.8 kg. Perineotomy and denervation of both kidneys.

21.1.49. Weight, 12.5 kg.

17.3.49. Weight, 14.0 kg.

29.3.49. Weight, 13.8 kg.

1.4.49. Killed.

M.58. Buster: Cross Bred Bull Terrier ♀

5.5.48. Denervation of kidneys. Weight, 13.6 kg.

11.5.48. Pituitary exposed. Perineotomy.
Weight, 13.6 kg.

12.5.48. Acute experiment. Acetyl choline injected into the supraoptic nucleus.

13.5.48. On heat.

10.6.48. Killed.

M.59. Wolf: Alsatian Type ♀

14.5.48. Weight, 18 kg. Denervation of both kidneys.

26.5.48. Died under anaesthesia while being operated on for gangrenous intussusception.

Dogs /

Dogs which were Observed during Recovery from the
Effects of D.F.P. Injected into the Supraoptic Nucleus

70. Spot: Small Terrier ♀

23.12.47. Perineotomy.

19.3.48- In metabolism cage.

2.4.48. Water drunk daily - average 30 c.c. (0 to 150 c.c.)

Urine passed daily - average 500 c.c. (620 to 735 c.c.)

7.4.48. Weight, 5.5 kg.

Total of 120 µg. D.F.P. injected into 3 sites in supraoptic nucleus.

Immediate inhibition of urine flow.

8.4.48- In metabolism cage.

27.4.48.

8.4.48- Water drunk - average 969 c.c. (1000 to 415 c.c.)

Urine passed - average 1278 c.c. (1800 to 1105 c.c.)

16.4.48- Water drunk - average 154 c.c. (320 c.c. to 20 c.c.)

27.4.48. Urine passed - average 5.40 c.c. (930 to 210 c.c.)

30.5.48- In metabolism cage.

5.6.48. Water drunk - average 4 c.c. (0 to 10 c.c.)

Urine passed - average 755 c.c. (845 to 550 c.c.)

16.12.48. Weight, 5.9 kg.

Killed under nembutal anaesthesia.

Histological Examination: Scar in hypothalamus on one side, running up beside wall of third ventricle. Other side - N.A.D.

Adenohypophysis - appears normal.

Neurohypophysis - ? a bit shrunken.

Supraoptic nucleus - cells appear normal.

Total cell count = approximately 16900.

Both rostral and caudal cells diminished in number.

Paraventricular nucleus - Total cell count = approximately 8435.

Scar runs through lower part of one nucleus.

72. Poppet: Small Terrier ♀

- 25.5.48. Weight, 6.6 kg. Both kidneys denervated.
- 27.5.48. Total of 100 ug. D.F.P. injected into 4 sites in supraoptic nucleus.
- 27.5.48- In metabolism cage.
- 30.5.48. Water drunk daily - average 500 c.c. (810 to 170 c.c.)
Urine passed daily - average 828 c.c. (725 to 965 c.c.)
- 31.5.48- water drunk daily - average 16 c.c. (0 to 5.6.48. 30 c.c.)
Urine passed daily - average 833 c.c. (670 to 805 c.c.)
- 16.12.48. Weight, 6.6 kg.
Killed under nembutal anaesthesia.

Histological Examination: No scar visible in hypothalamus.

Adenohypophysis - appears normal.

Neurohypophysis - appears normal.

Supraoptic nucleus - Total cell count = approximately 20890 cells with nucleoli, only 5245 of which had the normal number of Nissl granules.

Paraventricular nucleus - Total cell count = approximately 10790 cells. Mostly appear normal.

84. Snapper: Black and White Fox Terrier ♀

- 6.11.48 Perineotomy operation.
- 3.11.48- In metabolism cage.
- 14.11.48. Water drunk daily - average 102 c.c. (0 to 900 c.c.)
Urine passed daily - average 313 c.c. (0 to 617 c.c.)
- 30.11.48. Exposed pituitary under nembutal anaesthesia. Weight, 6.3 kg.
- 1.12.48. Total of 120 ug. D.F.P. in 3 doses into supraoptic nucleus. Immediate anti-diuretic effect.
- 1.12.48- In metabolism cage.
- 16.12.48. Water drunk daily - average 695 c.c. (12 to 1520 c.c.)
Urine passed daily - average 118 c.c. (553 to 1650 c.c.)
- 17.12.48 /

17.12.48- Water intake only recorded.
This varied between 95 and 700 c.c. per
24 hours.

85. Trix: Brown Terrier ♀

7.12.48 Denervation of both kidneys.
Weight, 8.62 kg.

14.12.48. Cleared pituitary site and perineotomy.

15.12.48. Total of 120 µg. of D.F.P. in 3 doses
20.12.48. into supraoptic nucleus. Immediate
antidiuretic effect.

15.12.48- Water drunk daily - average 1270 c.c.
20.12.48 (750 to 1490 c.c.)

20.12.48- Water drunk daily - average 30 c.c.
13.1.49. (10 to 60 c.c.)

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